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# Acta Medica Scandinavica

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**EDITORIAL**

# Atrial Fibrillation—Some Current Problems

The possibility of spontaneous reversion from long lasting atrial fibrillation to sinus rhythm is pointed out in this issue of the Acta Medica Scandinavica (16). A majority of the patients who have undergone such an unexpected development of their arrhythmia suffered from advanced rheumatic mitral valve disease. Even in modern textbooks rheumatic heart disease is sometimes indicated as one of the commonest causes of atrial fibrillation. This is, however, certainly not true any more in countries where rheumatic fever has declined dramatically with the introduction of antibiotics. In spite of the disappearance of rheumatic fever and thereby atrial fibrillation of this origin, atrial fibrillation is still one of our most common types of arrhythmias. Thus Ostrander et al. (17) found in 1959–60 atrial fibrillation in 2.3% of men and in 2.5% of women aged 60–69 years in Tecumseh, USA. In the population aged 70–79 the prevalence had increased to 3.0% in men and to 5.0% in women. Comparable figures have been found in Scandinavian population studies (however, only men are investigated!). Thus Jacobsson and co-workers (personal communication) report a prevalence of atrial fibrillation of 0.3% in men 50–59 years of age, 1.3% in the age group 60–69 and 4.8% in the age group 70–79. Wilhelmsen and co-workers (personal communication) found in 1963 no cases of atrial fibrillation in a representative subgroup of all men in Gothenburg born in 1913. Ten years later, however, a prevalence of 2.8% of atrial fibrillation was found in the same population—then 60 years of age.

### *Electrophysiological aspects*

There are good reasons to distinguish the different mechanisms by which atrial fibrillation may be initiated and maintained, respectively. An atrial ectopic beat can thus almost always be found as the phenomenon initiating atrial fibrillation (2). This mechanism is also well known from studies with programmed atrial stimulation. Thus, a stimulus in the "vulnerable phase" of the atrial muscle is capable of initiating atrial fibrillation even in completely healthy hearts! Once established, atrial fibrillation is a self-sustaining arrhythmia (13). The mechanism of self-sustaining fibrillatory-like arrhythmia in isolated segments of rabbit atrial myocardium has been elegantly demonstrated by Allessie et al. (1). As there are different mechanisms which may initiate and maintain atrial fibrillation, treatment can be directed in principle at preventing the one or the other.

Various electrophysiological mechanisms responsible for atrial fibrillation in man have been observed. Thus, a strongly accelerated atrial repolarization has been noted in hyperthyroidism (7). The resting membrane potentials of the atrial muscle cells are, however, not affected by the altered thyroid state. These are findings from experimental work in animals but are very likely applicable in man since hypothyroidism causes a prolongation of atrial repolarization (8). An accelerated atrial repolarization has also been demonstrated in patients with atrial fibrillation of other causes than hyperthyroidism and with poor prognosis concerning maintenance of sinus rhythm after DC conversion (5, 15). Contrary to the circumstances in hyperthyroidism, these patients are likely to have the accelerated repolarization as a consequence of a decreased resting membrane potential, as patients of this type have a significantly decreased content of potassium in the atrial myocardial cells (6).

It is likely that more exact knowledge of the precise electrophysiological mechanisms involved in initiation and maintenance of atrial fibrillation can lead to better alternatives than those of today in the treatment of the arrhythmia.

### *Prophylactic antiarrhythmic treatment*

It is generally not difficult to convert atrial fibrillation to sinus rhythm. Nowadays this conversion is almost invariably performed with the DC shock technique. Several studies have demonstrated an immediate conversion rate in the range of 90%. If the patient who is converted to sinus rhythm is not then treated with antiarrhythmic drugs, sinus rhythm can be expected to persist for only one year in 15–30% of the patients (3, 9, 20). Thus, con-

sistent prophylactic antiarrhythmic treatment is desirable after DC conversion of atrial fibrillation to sinus rhythm. In the above three studies ran domized technique demonstrated that patients who take quinidine after DC conversion to sinus rhythm maintain their sinus rhythm for a significantly long er time than those who do not take quinidine. Thus the number of patients in sinus rhythm in the quini dine group is approximately twice that in the place bo group one year after conversion to sinus rhythm. In spite of this demonstration that quinidine is prophylactically efficient after DC conversion of atrial fibrillation this mode of treatment has not been generally accepted. The main reason for this is the high incidence of gastrointestinal side-effects during quinidine treatment. In practice these side-effects mean that only two patients out of three can tolerate chronic prophylactic treatment. No other drug has demonstrated a statistically satisfactory efficacy regarding prophylaxis against relapse to atrial fibril lation up to one year after DC conversion to sinus rhythm from atrial fibrillation.

From the theoretical point of view amiodarone is an interesting drug for the patient group discussed above. As mentioned earlier the inability to main tain sinus rhythm is closely linked to an accelerated atrial repolarization. Amiodarone has the ability to delay atrial repolarization substantially. This has been demonstrated in vitro (19) and also in man (14). The efficacy of this drug in atrial fibrillation has not been demonstrated in controlled trials but anecdotal reports have favoured its use in this ar rhythmia (24). However this drug's side-effects—above all corneal opacifications and thyroid mal functions—have ruled out controlled trials with this indication.

Indications for conversion of atrial fibrillation to sinus rhythm are of course bound up with the suc cess of long term maintenance of sinus rhythm. There are several factors which can influence the persistence of sinus rhythm (21). Local traditions also have a bearing on the indications for DC con version of atrial fibrillation. With possibly improved pharmacological means to maintain patients in sinus rhythm the indications are likely to change in the future.

### *Atrial fibrillation and stroke*

Although atrial fibrillation is a common arrhythmia there are still several problems regarding the rou tine therapy. The relation between chronic atrial fibrillation and stroke is one example. In an autopsy material of 333 patients who had had atrial fibrilla tion (10) embolization was observed in 41% of those who had mitral valvular disease and in 35% of those with ischemic heart disease. In a control group of 58 autopsy patients without atrial fibrilla tion during their lifetime embolism was found in only 7%. The Framingham Study has also eluci dated the importance of chronic atrial fibrillation for the development of stroke (22). In 14% of all stroke cases an embolism was demonstrated. In cases with idiopathic atrial fibrillation stroke was 5.6 times as common as in an age matched population without atrial fibrillation. If the atrial fibrillation was a result of rheumatic heart disease stroke risk was in creased 18 times in both sexes compared with a sinus rhythm population! In chronic atrial fibrilla tion stroke appeared in a direct relationship to the duration of the atrial fibrillation.

Anticoagulant therapy has been applied very dif ferently in atrial fibrillation. There seems to be fairly good agreement that patients with rheumatic valvular disease and atrial fibrillation should be treated with anticoagulants. Opinions differ regard ing idiopathic atrial fibrillation. The risk of stroke may perhaps be diminished by more active treat ment in the way in which the patients are converted to sinus rhythm. In case attempts at DC conversion fail to restore sinus rhythm controlled trials with anticoagulants are needed to assess the possibilities to decrease the risk of stroke!

### *Control of ventricular rate*

The control of ventricular rate in atrial fibrillation is another question to be studied further. Digitalis treatment is the traditional way of adjusting ven tricular rate though $\beta$ receptor blockers and cal cium inhibitors may be used too (11, 12, 23). Heart rate at rest and during controlled physical exercise has mostly been used to evaluate atrioventricular conduction capacity during atrial fibrillation. Only a few reports have dealt with the ventricular rate dur ing atrial fibrillation over 24 hours. The goal of the treatment must be that the patient should have a ventricular rate that at any specific time is ideally adapted to the hemodynamic situation. In practice this can presumably be translated into the statement that a patient should have the same ventricular rate as if he had had sinus rhythm instead of atrial fibril lation.

*Atrial fibrillation in pre excitation*

Atrial fibrillation in association with pre-excitation is another specific problem It is well known that single patients with pre excitation will develop an extremely high ventricular rate during atrial fibrilla tion (4) An even higher ventricular rate may in some cases be induced by digitalis (18) Such a high ventricular rate may even proceed to ventricular fibrillation and immediate death Digitalis has been used for long periods in patients with pre excitation syndromes of different types—often with good anti arrhythmic effect In the light of the findings that digitalis may enhance atrioventricular conduction and thereby initiate ventricular fibrillation in select ed cases with accessory atrioventricular pathways it is advisable to abstain from acute or chronic digitalis treatment in patients with this anomaly un less an individual provocative test has demon strated a beneficial effect of the drug

With increasing age patients with pre excitation run of course the increased risk to develop atrial fibrillation as initially discussed The potential danger of atrial fibrillation in these patients has initiated provocative studies in patients who have re entry tachycardias as a consequence of their pre excitation (4) Perhaps even patients with pre-exci tations but without tachycardias who run an in creased risk of developing atrial fibrillation (for in stance due to age[11]) should be provoked to atrial fibrillation during controlled conditions in order to study the effective ventricular rate Only in such studies is it possible to evaluate whether atrial fibril lation may be deleterious for a patient with pre excitation and therefore necessitate prophylactic medical or surgical treatment

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## REFERENCES


1 Allessie M A Bonke F I M & Lammers W J E P The effects of carbamylcholine adrenaline oua bain quinidine and verapamil on circus movement tachycardia in isolated segments of rabbit atrial myo-cardium In Re-entrant arrhythmias—mechanisms and treatment (ed H E Kulbertus) chapter 5 MTP Press Lancaster 1977

2 Bennett M A & Pentecost B L The pattern of onset and spontaneous cessation of atrial fibrillation in man Circulation 41 981 1970

3 Byrne-Quinn E & Wing A J Maintenance of sinus rhythm after DC reversion of atrial fibrillation A double blind controlled trial of long acting quinidine bisulphate Br Heart J 32 370 1970

4 Campbell R W F Smith R A Gallagher J J Pritchett E L C & Wallace A G Atrial fibrillation in the preexcitation syndrome Am J Cardiol 40 514 1977

5 Cotoi S Gavrilescu S Pop T & Vicas E The prognostic value of right atrium monophasic action potential after conversion of atrial fibrillation Eur J Clin Invest 2 472 1972

6 Ebert P A Relationship of myocardial potassium content and atrial fibrillation Circulation (Suppl) II 137 1970

7 Freedburg A S Papp J G & Vaughan Williams E M The effect of altered thyroid state on atrial in tracellular potentials J Physiol 207 357 1970

8 Gavrilescu S Luca C Streian C Lungu G & Deutsch G Monophasic action potentials of right atrium and electrophysiological properties of AV conducting system in patients with hypothyroidism Br Heart J 38 1350 1976

9 Hillestad L Bjerkelund C Dale J Maltau J & Storstein O Quinidine in maintenance of sinus rhythm after electroconversion of chronic atrial fibril lation Br Heart J 33 518 1971

10 Hinton R C Kistler J P Fallon J T Friedlich A L & Fisher C M Influence of etiology of atrial fibrillation on incidence of systemic embolism Am J Cardiol 40 509 1977

11 Khalsa A & Olsson B Verapamil induced ven tricular regularity in atrial fibrillation Effects of exer cise isoproterenol atropine and conversion to sinus rhythm Acta Med Scand 205 509 1979

12 Khalsa A Olsson S B & Henriksson B Å Effect of oral verapamil on ventricular irregularity in cases of long standing atrial fibrillation Acta Med Scand 205 39 1979

13 Moe G K & Abildskov J A Atrial fibrillation as self sustaining arrhythmia independent of focal dis charge Am Heart J 58 59 1959

14 Olsson S B Brorson L & Varnauskas E Class 3 antiarrhythmic action in man Observations from monophasic action potential recordings and ami odarone treatment Br Heart J 35 1255 1973

15 Olsson S B Cotoi S & Varnauskas E Mono-phasic action potential and sinus rhythm stability after conversion of atrial fibrillation Acta Med Scand 190 381 1971

16 Olsson S B Örndahl G Enestrom S Eskilsson J Persson S Grennert M L & Johansson B W Spontaneous reversion from long lasting atrial fibrilla tion to sinus rhythm Acta Med Scand 207 5 1980

17 Ostrander L D Brandt R L Kjelsberg M O & Epstein F H Electrocardiographic findings among the adult population of a total natural community Tecumseh Michigan Circulation 31 888 1965

18 Sellers T D Bashore T M & Gallagher J J Digitalis in the pre-excitation syndrome Analysis dur ing atrial fibrillation Circulation 56 260 1977

19 Singh B N & Vaughan Williams E M The effect of amiodarone a new anti anginal drug on cardiac muscle Br J Pharmacol 39 657 1970

20 Sodermark T Jonsson B Olsson A et al Effect of quinidine of maintaining sinus rhythm after con

version of atrial fibrillation or flutter. Br Heart J 37: 486, 1975.
21. Waris E., Kreus K. E. & Salokannel J.: Factors influencing persistence of sinus rhythm after DC shock treatment of atrial fibrillation. Acta Med Scand 189: 161, 1971.
22. Wolf P. A., Dawber T. R., Thomas H. E. & Kannel W. B.: Epidemiologic assessment of chronic atrial fibrillation and risk of stroke. The Framingham Study. Neurology 28: 973, 1978.
23. Yahalom J., Klein H. O. & Kaplinsky E.: Beta adrenergic blockade as adjunctive oral therapy in patients with chronic atrial fibrillation. Chest 5: 597, 1977.
24. Zipes D. P. & Troup P. J.: New antiarrhythmic agents. Am J Cardiol 41: 1005, 1978.

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# Spontaneous Reversion from Long-Lasting Atrial Fibrillation to Sinus Rhythm

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**ABSTRACT** We have collected 23 cases with atrial fibrillation (AF) of 3-29 years' duration with spontaneous reversion to sinus rhythm (SR) We have also identified 22 similar cases in the literature Several of our patients spent several years with different atrial arrhythmias before a stable SR was established Of all the 45 cases 39 had significant rheumatic mitral valve disease In the majority of these patients the ECG shows first degree AV block after return to SR and a low amplitude P wave—as if the left atrial P component was lacking There are no signs of left atrial mechanical activity after re-establishment of SR in our mitral valve disease group, as judged from phonocardiograms, apexcardiograms, echocardiograms and left atrial pressure recordings in selected patients Heart muscle biopsy was obtained from two patients who underwent mitral valve surgery Left atrial specimens showed almost complete lack of all muscle structures There is thus electrical mechanical and histological evidence of left atrial muscle deterioration It is likely that the electrophysiological factors responsible for initiation and maintenance of AF have disappeared with this deterioration thereby allowing SR to be re-established The return of SR might indicate a progress of the heart disease although the patient may benefit from normalization of cardiac rate and regularity The easy identification of our 23 patients makes us believe that the phenomenon of appearance of late SR is far more common than suggested up to now

*Key words* atrial fibrillation sinus rhythm mitral valve disease

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Atrial fibrillation (AF) is usually classified as either paroxysmal or chronic (23 31) Paroxysmal attacks of AF sometimes tend to increase in duration and spontaneous reversion to sinus rhythm (SR) may ultimately fail resulting in chronic AF AF may cause haemodynamic deterioration an increased risk of embolism and subjective symptoms of varying severity When these effects are pronounced it is therefore desirable to convert AF to SR DC conversion has become the accepted treatment for this purpose

The likelihood of SR being maintained is however much reduced with increasing duration of AF (8 39) The majority of patients will ultimately relapse to AF after DC conversion even with aggressive antiarrhythmic prophylaxis (37) Chronic AF may thereafter be expected to accompany the patient for the rest of his life

However as long ago as in 1939 Burch (7) reported a case of AF lasting for two years and then spontaneously reverting to SR Including the case reported by Burch at least 22 cases of spontaneous reversion from AF to SR are described in the literature (5 11 12 15 18 19 21 27 32 42 44) Of these cases 21 are summarized in Table I one case is only briefly described by Saint Pierre et al (34) Nineteen of the 22 patients suffered from mitral valvular disease with or without concomitant aortic valve lesions In most cases a rheumatic origin was evident The serious functional deterioration observed in most of these patients is reflected in the increased heart size visible on radiographic examination Several of these patients had had AF for

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*Abbreviations* AF=atrial fibrillation SR=sinus rhythm AV atrioventricular ECG=electrocardiogram HR=heart rate BP=blood pressure ACG=apexcardiogram aML=anterior mitral valve leaflet PTAH=phosphotungstic acid haematoxylin

Table I *Reports from the literature on patients reverting from long standing atrial fibrillation to sinus rhythm*

NR=nodal rhythm PAT=paroxysmal atrial tachycardia SVT=supraventricular tachycardia MS=mitral stenosis MI=mitral incompetence AS=aortic stenosis AI=aortic incompetence LA=left atrium LV=left ventricle RV=right ventricle SA bl=sinoatrial block SB=sinus bradycardia Ao=aortic Mi=mitral

| Authors | Sex | Age at onset of late SR (y) | Duration of AF (y) | Diagnosis | Heart size (X ray) | Length of follow up of late SR | ECG in late SR |
|---|---|---|---|---|---|---|---|
| Burch (7) | ♂ | 63 | 1 10/12 | MI + syphilis | Heart enlarged | 18 mo (episode of AF) | SR |
| Fogel (12) | ♂ | 75 | 11 | Art scler heart dis | Normal | 4 mo | SR |
| Vaisrub (42) | ♂ | 47 | 3 | MS | LA enlarged | 1 mo | SR 1st degree AV block peaked narrow P |
| Lewis (27) | ♂ | 69 | 17 | Heart failure | LA enlarged | 9 mo → AF | SR 1st degree AV block peaked narrow P |
| | ♀ | 55 | 11 | MS | LA enlarged | 15 mo → AF | SR 1st degree AV block peaked narrow P |
| | ♂ | 73 | 11 | MS AS | Heart enlarged LA | 47 d → AF | SR |
| Halpern (18) | ♀ | 65 | 9 | MS MI AS AI | LV LA & RV enlarged | 14 mo (short burst of atrial flutter) | SR 40–60/min |
| Holtzmann (21) | ♀ | 67 | 28 | MI MS | Heart markedly enlarged | 13 mo | SR 40–57/min 1st degree AV block Broad flat P |
| | ♀ | 52 | 16 | MS MI | Heart markedly enlarged | 5 y (→5 y AF) | SR 105/min SA bl 1st degree AV block |
| Eichler (11) | ♀ | 76 | 8 | MS MI AI | – | 2 5 y | SR 1st degree AV block Broad flat P |
| Hanson & Tuna (19) | ♀ | 53 | 22 | MS op | LA enlarged | 4 mo | Not shown |
| Saint Pierre et al (34) | ♀ | 62 | 3 | MI | LA enlarged | 2 mo | SR RBBB |
| | ♀ | 57 | 3 | Ao & Mi prosth | LV LA & RV enlarged | 3 mo | SR 1st–2nd degree AV block |
| | ♂ | 39 | 3 5 | MS op c MI AI | Mitrale | 2 y | SR SB SA bl 1st degree AV block |
| | ♀ | 67 | 6 | Calc constr pericarditis | Calcif | 3 y | SR SA bl 1st degree AV block AF SVT pacemaker |
| Zimmerman et al (44) | ♀ | 47 | 12 | MS op | LA RV enlarged | 1 y | SR |
| | ♀ | 59 | 12 | MS op MI AI | Cardiomegaly | 2 y | SR 1st degree AV block PAT biphasic P in $V_{1-4}$ |
| | ♀ | 71 | 9 | MS op | – | 4 y | SR 1st degree AV block |
| Reeve et al (32) | ♀ | ? | 15 | MS op | – | – | SR S arrest pacemaker |
| Betriu et al (5) | ♂ | 50 | 10 (?) | MS op | – | 2 mo | SR 1st degree AV block |
| Froment et al (15) | ♀ | 56 | 13 | MS MI AI | Mitrale | 6 y | SR 1st degree AV block NR |

more than 10 years before reverting to SR A common finding during late SR is first degree AV block Only 3 patients all with different diagnoses were not afflicted with mitral valve disease

The phenomenon of unexpected spontaneous reversion to SR from long lasting AF has separately interested three groups of researchers from different universities in Sweden The purpose of this

Table II *Selected clinical details of Gothenburg patients reverting to sinus rhythm after several years of atrial fibrillation*

MS=mitral stenosis MI=mitral incompetence TI=tricuspid incompetence AS=aortic stenosis

| Case no | Sex | Born in | Diagnosis | Duration of AF (y) | Age at onset of late SR | Functional group (NYHA) | Heart size (total/m² BSA) |
|---|---|---|---|---|---|---|---|
| 1 | ♀ | 1904 | MS MI (op) | 17 | 61 | III | 1 350/800 |
| 2 | ♂ | 1903 | MS MI | >3 | 63 | III | 1 760/970 |
| 3 | ♀ | 1916 | MS MI TI | 15 | 55 | III | 2 100/1 450 |
| 4 | ♀ | 1912 | MS MI (op) AS (op) | >15 | 61 | IV | 1 450/940 |
| 5 | ♀ | 1896 | MS (op) | 12 | 72 | III | 1 150/700 |
| 6 | ♀ | 1909 | Lone AF Diab mell | 8 | 63 | II | 620/410 |
| 7 | ♀ | 1902 | MS MI (op) | 11 | 64 | III | 1 060/560 |
| 8 | ♂ | 1902 | MS MI | 15 | 72 | IV | 1 550/860 |
| 9 | ♀ | 1912 | MS MI TI | 29 | 64 | IV | 1 850/1 400 |
| 10 | ♀ | 1909 | MS MI AS | >6 | 63 | III | 990/680 |

paper is to analyse our altogether 23 additional cases in order to identify possible pathophysiological mechanisms involved in the observed changes in heart rhythm The clinical implication of the findings will also be discussed

## THE GOTHENBURG STUDY

### PATIENTS AND METHODS

The Outpatient Departments at Sahlgrenska Hospital serve patients with advanced cardiac diseases from a population of about half a million inhabitants From this patient pool 7 patients were observed over 10 years to revert from AF into SR after at least three years of AF Three more patients were identified from other sources Patients with long lasting AF who have been converted to permanent SR with quinidine or by DC conversion have been excluded from this study Selected clinical details of the 10 patients are given in Table II All earlier ECGs were sought in order to compare the late SR with earlier rhythms

ECGs recorded during SR before onset of AF could be traced for 3 patients (nos 5 8 and 10) Seven patients were treated with DC conversion or quinidine during the period of AF and all of them reverted to SR for short periods ECGs from these brief periods of SR have been identified for all 7 patients All ECG tracings were recorded at a paper speed of 50 mm/sec

Exercise tests with ECG recording were performed in 3 patients (nos 3 4 and 5) after they had reverted to SR The effect of i v isoproterenol (3 $\mu$g) and atropine (0 5 mg) on the rate and rhythm was also studied in one patient (no 5) Radiographic examination of the heart was performed in all cases within 4 months of reversion to SR

One patient (no 4) was subjected to right and transseptal left heart catheterization during late SR In 2 patients (nos 4 and 5) biopsy specimens from the left atrium were obtained for histopathological investigation during mitral valve surgery a biopsy from the right atrium was also obtained from patient 4 The sections were stained with periodic acid Schiff and with Mallory s phosphotungstic acid haematoxylin (PTAH)

## RESULTS

All but one of our 10 patients have had mitral valve disease with pronounced functional deterioration (Table II) Their heart volumes are all markedly enlarged except for the patient without mitral valve disease (no 6)

The duration of AF ranges between at least 3 and 29 years Seven of the patients were briefly converted to SR with quinidine or by DC conversion during the course of AF In none of them did this transient SR last for more than 5 months All patients then remained in AF for at least 5 years before late SR appeared All P waves recorded during the brief period of SR had a mitral configuration although the left atrial component was consistently of low amplitude Two of these recordings are depicted in Fig 1

One patient (no 9) exhibited transient rhythms of types other than AF *before* late SR ultimately stabilized Thus she had atrial tachycardia with a rate of 200/min AV block and nodal escape rhythm for several years This type of arrhythmia also occurred in 4 additional cases *after* the late SR had appeared

The ECG during late SR shows a uniform pattern in most of the 9 patients with mitral valve disease (Table III) Thus all of them had first degree AV block and several had P waves of low amplitude

Table III *Transient rhythms before late SR ECG analyses and stability of late SR in Gothenburg patients*
LAH = left anterior hemiblock, VEBs = ventricular ectopic beats SB = sinus bradycardia, SVEBs = supraventricular ectopic beats AT = atrial tachycardia, NR = nodal rhythm NEB = nodal escape beat AMI = acute myocardial infarction

| | | Late SR | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Case no | Transient rhythms before late SR | HR (beats/min) | PQ (sec) | P dur (sec) | P amp (mV) | P wave visible in lead | Follow-up | Stability |
| 1 | SR 2 d after quinidine treatment in 1959 | 50 | 0 25 | 0 12 | 0 15 | $V_1$–$V_4$ II–III | 1 mo († mes thromb ) | Late SR → AF after 1 mo died 1 d later |
| 2 | – | 48 | 0.29 | 0 09 | 0 25 | All (LBBB) | 5 y († unknown cause) | Alternated between AT with AV block and SR for 5 y |
| 3 | SR 1 d after DC conv in 1964 | 70 | 0.29 | 0 11 | 0 20 | $V_1$–$V_2$ II | 6 y | Mostly SR but also AT with AV block SB with NEB and NR |
| 4 | SR 1 d after repeated DC conv in 1965 | 92 | 0.23 | 0 11 | 0 40 | All (LAH) | 4 y | SR alternating with episodes of AT with 2 1 AV block and AF SR stable after 4 mo |
| 5 | – | 67 | 0.26 | 0 09 | 0 15 | $V_1$–$V_2$ | 9 y († heart failure) | SR alternating with AF AT with block during 3 y then SR with occasional SVEBs |
| 6 | SR 3 d after quinidine treatment in 1964 | 79 | 0 19 | 0 08 | 0.20 | All but AVL | 4 y | Stable SR |
| 7 | Short episodes of SR after DC conv in 1961 | 72 | 0 34 | 0 07 | 0 10 | $V_1$–$V_2$ (VEBs) | 7 y († stroke) | SR with occasional VEBs |
| 8 | SR 4 h after DC conv in 1966 | 64 | 0 37 | 0 12 | 0 20 | All but aVL | 2 y († AMI) | Daily fluctuation between SR and AF |
| 9 | AT with block and NR in episodes during 3 y before late SR | 58 | 0 19 | 0 07 | 0 05 | $V_1$–$V_2$ | 4 d → AF | SR for short periods otherwise AF or AT with block |
| 10 | Repeated brief episodes of SR after DC conv 1967–1968 | 84 | 0 24 | 0 08 | 0 25 | All | 3 y († stroke) | SR mostly AF on rare occasions |

The P wave duration ranged between 0 07 and 0 12 sec the exact duration was difficult to measure in several cases owing to the low amplitude

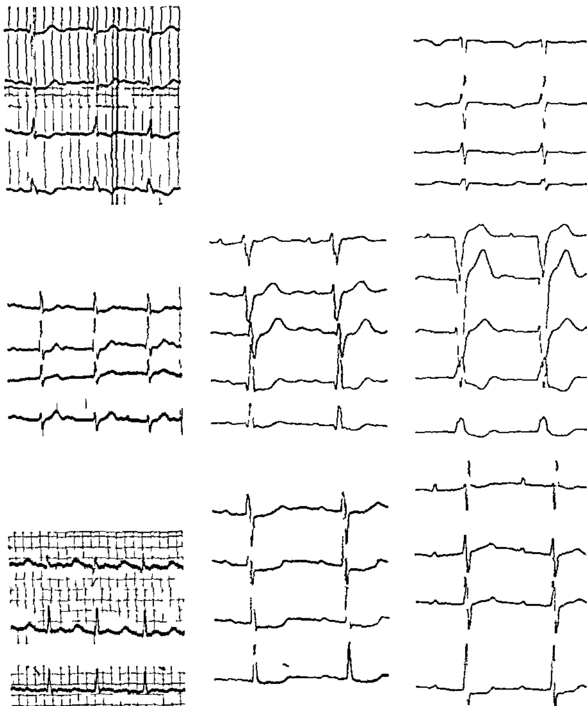
The stability of the late SR varied (Table III) Thus one patient (no 5) maintained regular SR without any other rhythm for 9 years until she died of cardiac failure Other patients never established stable SR but alternated spontaneously between SR and other rhythms

ECGs recorded during SR before AF had started were traced for three patients (nos 5 8 and 10) These are presented together with examples of transient SR after DC conversion and late SR in Fig. 1 All of them initially had P waves indicating left atrial enlargement

Physical exercise resulted in increased heart rate (HR) in the 3 patients who were subjected to ergometer tests (Table IV) HR also increased from 72 to 84/min after i v isoproterenol (case 5) and to the same level after i v atropine

Table IV *Effect of exercise on heart rate during late sinus rhythm in Gothenburg patients*

| Case no | Resting HR (beats/min) | Exercise load (W) | Exercise HR (beats/min) |
|---|---|---|---|
| 3 | 55 | 30 | 87 |
| 4 | 72 | 70 | 98 |
| 5 | 62 | 15 | 115 |



*F g 1* ECG record ngs f om Gothenburg pat ents 5 8 and 10 (top m ddle and bottom set of ECGs respect ve ly) durıng SR before onset of AF (to the left) durıng trans ent SR after DC convers on (cases 8 and 10 only) and durıng spontaneously occurrıng SR after many years of AF (to the rıght) Note the class cal m ral conf gurat on of the P wave durıng early and ın ermed ate transıent SR and the smaller and narrower P wave durıng late SR All record ngs show precord al leads

Table V *Clinical details of Lund patients with spontaneous return of sinus rhythm after long standing atrial fibrillation*

MS=mitral stenosis MI=mitral insufficiency AS=aortic stenosis AI=aortic insufficiency

| Case no | Duration of AF (y) | Sex | Age at sponteneous return of SR (y) | Diagnosis | Follow up | AV conduction (sec) | Heart volume (ml) (total/relative) |
|---|---|---|---|---|---|---|---|
| 1 | 15 | ♀ | 60 | MS | SR on one occasion later AF | P Q 0 28 | 1 250/720 |
| 2 | 17 | ♀ | 64 | MS | Alternating sinus and nodal rhythm during a couple of weeks later AF After 3 y slow nodal rhythm requiring pacemaker | P Q 0 20–0 22 | 1 240/760 |
| 3 | 13 | ♀ | 59 | MS + MI | SR on one occasion then atrial tachycardia | P Q 0 32 | 1 500/880 |
| 4 | 11 | ♀ | 75 | MS + AI | See text | P Q 0 22 | 1 050/680 |
| 5 | 10 | ♀ | 58 | MI + MS | See text | P-Q 0 16 | 1 110/780 |
| 6 | 3 | ♀ | 77 | AS + AI + MI | SR 6 mo later alternating SR AF atrial tachycardia Died 3 1/2 y later | P Q 0 26–0 30 | 1 560/930 |
| 7 | 7 | ♂ | 61 | MS | Episodes of atrial tachycardia and nodal rhythm otherwise SR until death 9 y later | P Q 0 18–0 24 | 1 180/685 |
| 8 | 8 | ♀ | 66 | MI + MS + AI | See text | P Q 0 22–0 24 | 1 840/1 090 |
| 9 | 8 | ♀ | 68 | Idiopathic AF | See text | P Q 0 20 | – |

* Estimated from a frontal and lateral erect chest X ray in most cases within one year after the return of SR Relative heart volume is expressed in ml/m² BSA

One patient (no 4) who was catheterized as part of a routine preoperative evaluation had no a waves in the left atrial pressure curve whilst the right atrial pressure tracing had a waves of normal amplitude Another possible sign of mechanical activity of the left atrium presystolic thrill was absent in all patients with other signs of mitral valvular obstruction

Microscopic examination was performed in two cases presented below

*Case 4*

Biopsies from the right and left atria (Figs 2 and 3) showed quite different histological appearances The *right atrium* had enlarged pectinate muscles of well preserved but hypertrophic fibres arranged in a loose network The myofibrillary striation looked normal—there were no signs of contraction bands necrosis or nuclear pyknosis Very slight interstitial fibrosis but no focal scarring was seen The endocardium was slightly thickened The *myocardial changes thus corresponded to grade I according* to Bailey et al (3) The *left atrial* wall was very severely damaged The myocardial fibres were almost completely replaced by dense collagenous bundles in continuity with a greatly thickened sclerotic epicardium The fibrotic scar tissue enclosed irregularly distributed fragmented muscle fibres which were vacuolated or granulated with loss of striation The nuclei were small and pyknotic There were also focal fatty changes in the stromal tissue but no inflammatory reaction The endocardium showed fibrotic thickening The myocardial damage was thus of grade III according to the classification of Bailey et al i e almost complete fibrosis

Microscopically the extensive degeneration of muscle fibres with fibrous replacement in the left atrium strongly resembled a healed infarct The diffuse scarring without cellular infiltrations seemed to distinguish this process from chronic myocarditis

*Case 5*

The biopsy from the left atrium had large pectinate muscles which appeared to be hypertrophic having thick muscle fibres with large rectangular nuclei Many small focal fibrotic scars were seen especially in intertrabecular muscles often in continuity with considerably thickened epicardium Interstitial fibrosis but no inflammatory reaction was seen The endocardium showed fibrotic thickening The myocardial changes thus corresponded to grade

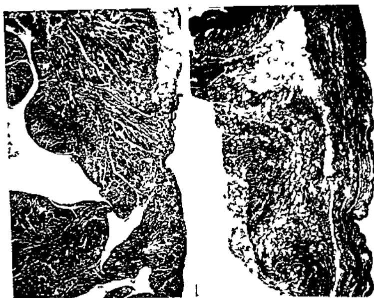


*Fig 2* Gothenburg case 4 (Left) Survey micrograph from the *right* atrial wall showing enlarged pectinate muscles well preserved muscle bundle architecture and very slight interstitial fibrosis (Right) In comparison to the right atrial wall the *left* atrial wall shows severe damage with unrecognizable architecture loss of muscle mass and fibrous tissue replacement (red) Greatly thickened sclerotic epicardium (to the right) (PTAH ×20)



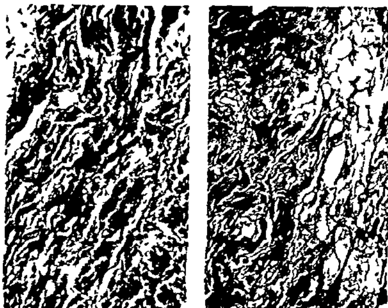


*Fig 3* Gothenburg case 4 (Top) *Right* atrial myocardium ordinarily arranged in the form of a loose network of well stained (violet) slightly hypertrophic muscle fibres (Bottom left) *Left* atrial wall showing dense bundles of collagenous fibres (red) enclosing fragments from a completely disrupted and atrophic myocardium (violet) (Bottom right) *Left* atrial myocardium with remnants of muscle fibres (violet) in collagenous fibrous tissue (red) focally rich in fat cells (white) No inflammatory cell infiltrates (PTAH ×160)

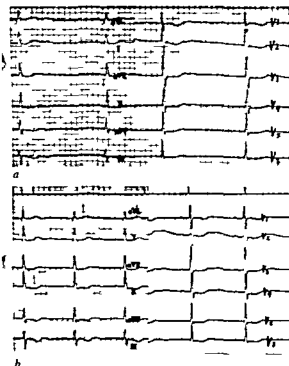


*Fig. 4.* Lund case 5. (*a*) ECG from Oct. 1975 showing AF with fine fibrillatory waves. (*b*) ECG from Oct. 1976 after return of SR.

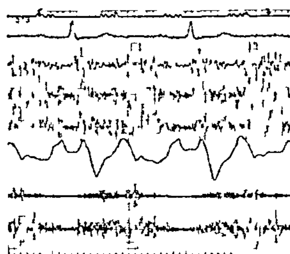


*Fig. 5.* Venous pulse curve from Lund case 8 after return of SR, July 1977, showing a normal a wave.

II according to Bailey et al. (3). The small fibrotic foci les ons situated near destroyed arteriolum nal channels suggested a correlation between chronic epicarditis and myocardial scarring in this case.

## THE LUND STUDY

During the last decade nine patients who spontaneously reverted to SR after 3-17 years of AF have been seen in Lund (Table V). In four of these patients non invasive investigations—including registration of apexcardiograms (ACG), venous pulse curves and echocardiograms—were performed. These patients are presented below in more detail.

## CASE REPORTS

*Case 4*

Woman born in 1901 with mitral stenosis and moderate aortic insufficiency diagnosed in 1964. AF appeared in 1965 and an attempt to DC-convert the AF to SR was unsuccessful. During the following years the patient was seen regularly once or twice a year. She had no cardiac symptoms at rest although her working capacity was reduced. In March 1976 her ECG showed AF with fine fibrillation waves. In June 1976 she was found to be in SR which has persisted since then. There has been no change in the patient's clinical state since the return of SR although she had noticed that her heart beats more regularly. In July 1977 non-invasive investigations were carried out. An a wave was found in the venous pulse curve but could not be demonstrated in the ACG. The echocardiographic findings indicated a severe mitral stenosis with a dilated left atrium. The rate of the early diastolic posterior motion of the anterior mitral valve leaflet (aML) was 15 mm/sec with an opening amplitude of 1? mm.

*Case 5*

Woman born in 1918 with mitral valvular disease diagnosed around 1930. AF developed in 1960. On a constant digitalis dose the patient remained essentially free from cardiac symptoms. In 1975 an episode of slow nodal rhythm was observed. Otherwise AF with fine f waves continued until Oct. 1976 (Fig. 4*a*) when an ECG showed SR with positive P waves in lead V (Fig. 4*b*). During the following months the patient developed congestive heart failure while still in SR. Signs of tricuspid insufficiency were also noted. She improved on diuretics and has since had no cardiac symptoms at rest or during light exercise. Non invasive investigations were performed in Aug. 1977. An a wave was found in the venous pulse curve but not in the ACG. On the echocardiogram the rate of the early diastolic posterior motion of the aML was 37 mm/sec and the opening amplitude was 20 mm, i.e. compatible with mild mitral stenosis. No A wave could be found in the motion pattern of the aML. However, a normal A wave was found in the motion pattern of the anterior tricuspid valve. The left atrium was markedly dilated to 60 mm.

*Case 8*

Woman born in 1901 with mitral valvular disease and aortic regurgitation. AF developed in 1959 and continued until 1967, when SR spontaneously returned. At regular

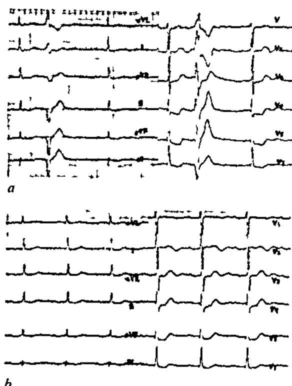


*Fig 6* Lund case 9 (*a*) ECG from June 1976 showing AF with fine fibrillatory waves (*b*) ECG from Feb 1977 after return of SR

examinations during the succeeding five years the ECG showed regular SR but in 1972 atrial tachycardia with 2 1 AV block was observed Since then the patient has had SR with diphasic P waves in lead $V_1$ alternating with atrial tachychardia and occasional periods of AF with coarse f waves in lead $V_1$ Throughout the years the patient has shown no signs of cardiac decompensation on digitalis and diuretics Repeated controls of her plasma digoxin levels have revealed no signs of digitalis intoxication and there has been no correlation between the dose of digoxin and the appearance of atrial tachycardia In July 1977 an ECG showed SR with first degree AV block (P-Q 0 24 sec) The venous pulse curve showed a normal a wave (Fig 5) but a distinct a wave could not be found in the ACG At echocardiography no A wave appeared in the motion pattern of the aML A hugely dilated left atrium was found (65-70 mm) while the degree of mitral stenosis was estimated as mild (the rate of early diastolic posterior movement of the aML 32 mm/sec opening amplitude 18 mm)

*Case 9*

Woman born in 1908 Mastectomy for carcinoma of the breast in 1954 In 1968 AF developed An attempted DC conversion some months later was unsuccessful The patient had no signs of valvular disease and her fibrillation was considered to be essential Since then she has been seen as outpatient twice yearly On a constant dose of digitalis she has been free from cardiac symptoms Cutaneous metastases of the breast cancer have been known to be present since 1973 In June 1976 the ECG still

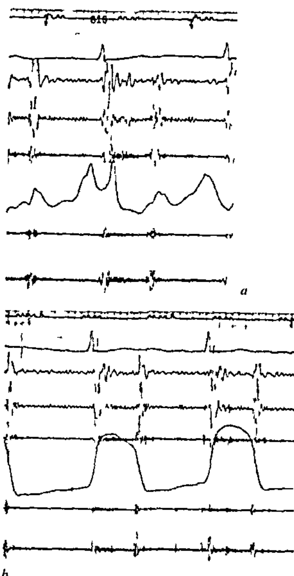
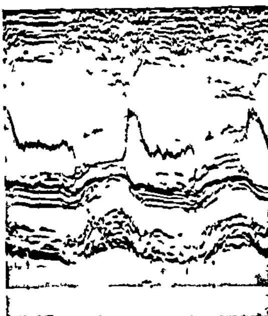
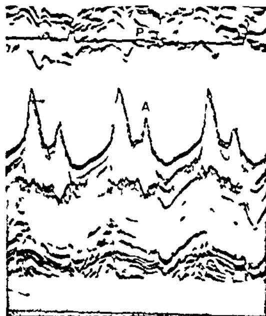


*Fig 7* Lund case 9 (*a*) Venous pulse curve during SR in Oct 1977 showing a distinct a wave (*b*) ACG during SR in Oct 1977

showed AF (Fig 6*a*) Some months later the patient noticed that her heart beat more regularly and another ECG in Feb 1977 showed SR with normal AV conduction (Fig 6*b*) The SR has persisted since that time In Oct 1977 the venous pulse curve showed a normal a wave (Fig 7*a*) but no distinct a wave could be distinguished in the ACG (Fig 7*b*) No signs of mitral stenosis could be found on echocardiography The left atrium was moderately enlarged (51 mm) The motion pattern of the aML was abnormal with almost complete disappearance of the A wave (Fig 8)

## THE MALMO STUDY

Four patients who spontaneously reverted to SR after AF for 3-10 years have recently been seen in Malmo


*F g 8* Echocard ogram from Lund case 9 during SR n Oct 1977 (left) compared to a normal echocard ogram (right) The mot on pattern of the aML shows a d m nut ve A wave but is otherwise normal

*Case 1*

This pat ent s a woman born n 1903 w thout known rheumat c fever Her d abetes mell tus d agnosed n 1961 is well controlled by chlorpropam de 0 25 g da ly In 1957 she f rst experienced attacks of palp tat on and was g ven d g tal s owing to moderate symptoms of card ac decom pensat on In 1967 chlorthal done was added In the early 1960s phys cal f nd ngs ind cated a valvular heart les on and in 1973 a def n te d agnos s of m tral stenos s was conf rmed by an accentuated f rst heart sound and an open ng snap In add t on an ap cal pansystol c med um p tched murmur of grade 2 (scale 1–6) was heard In 1975 a BP of 200/100 mmHg was recorded and she was given propranolol in a dose of 10 mg t i d As no reduct on of the BP was observed n April 1975 sp ronolactone 50 mg b i d was added to the prev ous med cat on w th lanato-s de C 0 25 mg t d and chlorthal done 50 mg once da ly

In March 1967 an ECG showed AF No cl n cal s gns of thyrotox cos s were observed and thyro d funct on tests (prote n bound iod ne and $T_3$) were normal In 1968 the pat ent was DC-converted but AF recurred two days later During the subsequent years ECGs were recorded annually all show ng AF with low f wave ampl tude In Aug 1976 a general pract t oner noted a b gem ny and one month later regular rhythm No ECG was recorded unt l Jan 1977 and it showed SR w th f rst degree AV block P R 0 28 sec A chest X ray in 1968 showed a relat ve heart volume of 470 ml/m² BSA (29)

*Case 2*

Th s patient a woman born in 1898 was hem thyro dec tom zed n the 1930s ow ng to non tox c go tre D abetes mell tus d agnosed at the age of 61 s well controlled with d et No cl nical s gns of thyrotox cos s have been observed and thyro d funct on tests (prote n bound od ne and $T_3$) were normal in 1969 and 1972 In Aug 1976 there were st ll no cl n cal s gns of thyrotox cos s but $T_3$ and T values were at the upper l m ts of the normal ranges In 1965 she experienced palp tat on In 1967 an ECG showed AF

A d agnosis of m tral insuff ciency was based on a med um p tched pansystol c ap cal murmur grade 3 DC convers on n 1969 resulted n s nus bradycard a and AV d ssoc at on Although d g tal s and qu n d ne (g ven after electroconvers on) were w thdrawn the s nus bradycard a pers sted She was interm ttently g ven atrop ne 0 25 mg 2 3 times da ly and orc prenal ne ?0 mg 3–4 t mes da ly with no clear-cut effect on the HR In Feb 197? AF recurred Electroconvers on was performed tw ce during the spring of 1972 but AF recurred on both occas ons after a short t me

Although she was on d goxin 0 25 mg da ly moderate card ac decompensat on developed and d uret cs were g ven (trichlormeth az de 4 mg four days a week later reduced to once a week) Repeated ECG record ngs showed AF w th low f waves unt l Jan 1976 when SR occurred The P R durat on was 0 35 sec w th periods of s nus bradycard a 40/m n and AV d ssoc at on She compla ned of vert go wh ch was ascribed to the bradycard a and was therefore g ven orc prenal ne w th a moderate effect on the vert go and HR wh ch rose from 40 to 48–50/m n An ECG in June 1976 showed pre exc ta t on w th P R 0 04 sec S nce Aug 1976 she has had AF w th a h gh ventricular rate 150–1?0/m n

A chest X ray n 1968 showed a sl ghtly enlarged heart

with a relative volume of 590 ml/m² BSA This volume has remained unchanged at subsequent examinations in 1969 1972 1973 1974 and Jan 1976

*Case 3*

This patient is a woman born in 1904 with no history of rheumatic fever Congestive heart failure developed in 1967 and was treated with digitalis and diuretics At the same time AF was observed on the ECG There were no clinical or laboratory findings indicating thyrotoxicosis A slight hypokalaemia was successfully treated with potassium supplements Chronic dicoumarol treatment was initiated in 1971 owing to relapsing venous thrombosis in her legs Diabetes mellitus diagnosed in 1975 is well controlled by chlorpropamide 250 mg daily her kidney function and BP are normal In 1975 mitral stenosis was diagnosed based on an accentuated 1st sound opening snap and a rumbling grade I diastolic apical murmur

The ECG has been recorded twice a year since 1967 showing each time AF and f waves of low amplitude until May 1977 when SR with infrequent supraventricular premature beats was found No change in the patient s clinical condition was observed when SR appeared Chest X rays have shown a constant heart volume over the years total 1 240 ml and relative 730 ml/m² BSA in April 1967 the corresponding values in Oct 1967 being 1 000 and 630 and in June 1977 1 050 and 700

*Case 4*

This patient is a man born in 1904 An elevated BP was noted in the 1950s but not treated until 1972 when he suddenly developed congestive heart failure He had AF on admission to hospital and gout was diagnosed An elevated BP (200/100 mmHg) normalized after treatment with methyldopa 0 25 g q i d and a low salt diet He is also on 40 mg of furosemide daily and potassium supplements

He has had moderate hepatomegaly his clinical condition has been good and his BP was 190/85 mmHg in Aug 1977 There were no signs of valvular heart disease or diabetes mellitus until Aug 1978 when a raised blood glucose of 15 mmol/l was noted

Since 1972 repeated ECGs have shown AF with f waves of low amplitude until 1976 when SR 58/min with first degree AV block appeared (P R 0 25 sec)

A chest X ray in 1972 showed slight enlargement of the heart with a total volume of 930 ml and a relative volume of 510 ml/m² BSA The corresponding figures in 1977 were 1 100 and 600

## DISCUSSION

Spntaneous reversion from long standing AF to SR is rare In our patients the duration of AF preceding spontaneous SR was at least 3 years and in most cases more than 10 years

All patients were followed for long periods AF appeared regularly on the ECG recordings and since the appearance of SR its persistence was repeatedly confirmed electrocardiographically The likelihood of the occurrence of short bouts of SR during the long period of AF is therefore very small The persistence of the AF was confirmed by the patients subjective experience although not all could reliably state when their heart rhythm had changed

Our 23 cases confirm the reports in the literature inasmuch as a distinct group of patients reverting from long lasting AF to SR can be identified A long lasting and severe mitral valve disease is a predominant finding in our patients (20 of 23) as well as in the cases reported in the literature (19 of 22) The other 6 patients (3 from our series and 3 from the literature) were all suffering from different types of heart disease and the only clinical feature they appear to have in common is that they reverted unexpectedly from long lasting AF to SR

Changes in medical treatment deterioration of ionic balance or concomitant disease may sometimes explain a reversion to SR during AF These factors have been ruled out in all our cases and the reason why these patients ultimately reverted to SR can only be speculated upon The observation that 3 out of 4 patients in the Malmo group 2 in the Lund group and one in the Gothenburg group suffered from manifest diabetes during the reversion and the fourth Malmo case later developed diabetes may suggest that microangiopathy or peripheral (cardiac) nervous damage may play a role in the development of late SR Two of the 4 Malmo patients were rather heavy alcohol consumers further supporting the possibility of nervous damage However none of them showed any clinical signs of peripheral neuropathy nor were there any signs of retinopathy or nephropathy Finally diabetes is not a common finding among our other cases nor does it appear to be an important factor regarding the cases reported earlier

It may be argued that patients not suffering from any specific chronic heart disease may develop AF as a result of a transitory cardiac damage for instance myocarditis and may then maintain a permanent AF owing to the self sustaining properties of this arrhythmia However DC conversion after the onset of AF was either unsuccessful or resulted only in transient SR in most of our patients without signs of mitral valve disease

All our patients with mitral valvular disease have had a long lasting and incapacitating rheumatic mitral valvular disease The disease has often been severe enough to warrant surgical treatment The

majority of these patients have had AF for more than 10 years before late SR appeared

*Electrophysiological aspects*

Analysis of ECGs recorded during AF reveals that some patients have f waves of rather low amplitude It may be questioned whether the observed rhythm in fact has been atrial stand still and nodal escape rhythm We have met this problem in several patients with spontaneous reversion to SR If however there has not been a clearly definable P wave in at least two leads we have excluded the patients from this report It is in fact possible that such patients have a rhythm which is generated from structures in the atrium—probably sinus node—and have a concealed conduction to the AV node (14)

Patients with rheumatic mitral valvular disease and AF usually have coarse fibrillatory waves An f wave amplitude of more than 0 5 mm has been reported in 63–89% (1 2 41) Using an amplitude of 1 0 mm as the borderline between coarse and fine waves Peter et al (30) found coarse waves in 85% of a group of patients with rheumatic heart disease It has been suggested that the condition of the atrial tissue is the most important factor influencing the f wave amplitude and that the extent of the inflammatory reaction in rheumatic heart disease is probably of utmost importance (1) This is consistent with the clinical impression that large amplitude fibrillation is more common in recent fibrillation (35) The f wave amplitude in 6 of the 8 Lund patients with mitral valvular disease was less than 1 0 mm A degenerated left atrial tissue is a possible explanation of this finding

In the Malmo group AF before SR was initially characterized by coarse f waves These diminished with increasing duration of AF and digitalis treatment The possibility that this reduction of f wave amplitude might be due to a reduction of the volume of the left atrium (41) could not be confirmed by X ray volume determinations

The P wave is always of unusual shape during late SR in the mitral valvular disease group However the vector of the P waves is initially the same as that of normal SR indicating that the origin of the rhythm is at least in the upper part of the right atrium

The conduction velocity atrial myocardial thickness and atrial size are factors which all influence the P wave configuration Of these factors only atrial size is possible to assess in our patients This factor alone cannot however help us to explain the appearance of the P wave during late SR This is often of low amplitude as well as of moderate duration This specific type of P wave is however documented in cases of atrial dissociation with independent right and left atrial rhythms (40)

A direct comparison can be made between the appearance of the P wave before AF during transient SR after DC conversion and during late SR in several of our cases and in some cases in the literature (11 27 34) Such a comparison reveals that the P wave component attributed to left atrial depolarization has usually disappeared when late SR is established This component was however present in all our cases in the ECG recorded before AF first appeared as well as in the ECGs recorded during the brief periods of SR after DC conversion

Patients with mitral valvular disease in SR generally have a mitral P with a predominant negative terminal portion of the P wave in lead $V_1$ thought to be caused by the depolarization of a hypertrophic left atrium (30) In the Lund series this negative terminal portion was missing in those six patients who had fine fibrillatory waves before the conversion thus supporting the concept of a degenerated left atrial myocardium The remaining two Lund patients on the other hand had coarse f waves and the classical pattern of mitral P when in SR

We have documented an increase in HR during physical exercise as well as after atropine and isoproterenol in a few patients during late SR supporting the view that the rhythm is generated by the sinus node

It is tempting to conclude from the different observations cited above that the P wave observed in late SR is due to electrical activity in the right atrium only

Thus it seems justified to suggest that progressive atrial tissue degeneration might influence atrial electrophysiology Further evidence of this is given by Khan et al (26) who describe two patients with advanced mitral valvular disease whose surface ECGs showed total absence of atrial activity Intracardiac electrograms showed SR in both cases The authors suggest that extensive atrial myocardial fibrosis caused the sinus impulse to travel through the internodal tracts without depolarizing the atria The absence of atrial mechanical activity was demonstrated in one of the patients by pressure

hypertensive heart disease was diagnosed in three of the patients It is conceivable that even small added microangiopathic changes due to diabetes might be enough to produce symptoms in such a heart

If this microangiopathy plays a role in the patients reported here it might do so in two ways Firstly fibrosis of the myocardium might be produced resulting in interruption of the re-entry pathways Secondly the nutrition of the autonomous nerves could be interfered with Several studies have displayed evidence of vagal denervation of the heart in established diabetes (28 43) A recent study showed that autonomic dysfunction might be present at the time of diagnosis of diabetes mellitus (13)

Vagal stimulation shortens the refractory period of the myocardium thereby favouring the precipitation of AF On the other hand decreased vagal tone prolongs the refractory time and shortens the conduction time making AF less easy to induce Normally the vagal tone decreases with increasing age

In normal subjects complete pharmacological blockade of the autonomic nervous system of the heart with propranolol and atropine induces an increase in HR, the magnitude of the increase being less with increasing age On the other hand blockade was found to lower the HR in patients with ischaemic valvular or congenital heart disease the decrease paralleling the severity of the cardiac disease (25) Our patients do not display a high resting HR which would be the case if vagal dysfunction existed The HR at rest tends rather to be low This could be explained by a concomitant sympathetic dysfunction in addition to the parasympathetic one

*Clinical implications*

We thus have ECG mechanical and histopathological evidence that the left atrial muscle is inactive during late SR in patients with mitral disease In contrast to these findings at least one of the three patients without mitral valve disease and spontaneous reversion to late SR had a P wave of completely normal appearance An apparently normal P wave can also be seen in recordings from two of the four patients without mitral valve disease and with long standing but self limiting AF reported by others (7 12) It is therefore likely that the electrophysiological cause of the AF in these patients differs from the mechanism discussed above

Furthermore these observations do not support the assumption that long standing AF in itself leads to structural atrial changes thereby making the AF irreversible (3)

It might be concluded from our findings that some patients with severe mitral valve disease have AF only within the right atrium These patients might be able to maintain SR after DC conversion thus also being able to benefit from the possible advantages of this late SR

Although SR may ultimately appear in some patients with severe mitral valve disease they can only benefit from some of the advantages of SR—the normal variability of the rhythm and the ventricular regularity The mechanical atrial activity is absent and therefore also the rise in cardiac output that would normally be expected when SR is restored (33) In addition the risk of intra atrial thrombus formation is presumably not diminished In fact the incidence of atrial embolism seems to be high during late SR in our series as well as in the literature Anticoagulant therapy should therefore not be discontinued when late SR appears

The return of SR after many years of AF does not necessarily indicate recovery it might rather indicate further damage to the patient although no changes in the clinical condition are observed It is obvious that despite spontaneous return of SR the ECG findings of first degree AV block AV dissociation and bradycardia indicate the severity of the remaining heart disease

We have easily identified 23 cases of late SR in the population served by three medium sized Swedish university hospitals altogether amounting to about 2 5 million inhabitants It is likely that several more cases exist—patients treated by other doctors and patients overlooked by us Bearing these facts in mind the total number of cases reported in the literature is very small We believe that the appearance of late SR is a far more common phenomenon than suggested up to now

## REFERENCES

1 Åberg H Coarse and fine atrial fibrillation method of evaluation relation to mechanism and aetiology of fibrillation In Symposium on Cardiac Arrythmias Elsinore Denmark p 47 Astra Sodertalje 1970
2 Aravanis C Toutouzas P & Michaelides G Diagnostic significance of atrial fibrillatory waves Angiology 17 781 1966

3 Bailey G W Braniff B A Hancock E W & Cohn K E Relation of left atrial pathology to atrial fibrillation in mitral valvular disease Ann Intern Med 69 13 1968

| Benchimol A & Desser K B Clinical applications of the apexcardiogram In Zoneraich S Non invasive methods in cardiology p 101 Thomas Springfield 1974

i Betriu A Sanz G Adelman A G & Wigle E D Sinus rhythm with ineffective left atrial contraction in severe mitral stenosis Chest 66 441 1974

5 Biggs F D Lefrak S S Kleiger R E Senior R M & Oliver G C Disturbances of rhythm in chronic lung disease Heart Lung 6 256 1977

7 Burch G Auricular fibrillation of twenty two months duration with return to normal sinus mechanism without the aid of quinidine Am Heart J 18 102 1939

3 Cramer G Early and late results of conversion of atrial fibrillation with quinidine Acta Med Scand (Suppl) 490 1968

) Davies J & Pomerance A Pathology of atrial fibril lation in man Br Heart J 34 520 1972

) Ebert P A Relationship of myocardial potassium content and atrial fibrillation Circulation (Suppl) II 137 1970

1 Eichler B B Spontaneous reversion of perma nent atrial fibrillation to regular sinus rhythm An giologia 18 476 1967

2 Fogel M Auricular fibrillation of long standing with spontaneous return to normal sinus rhythm Am Heart J 25 700 1943

3 Fraser D M Peripheral and autonomic nerve func tion in newly diagnosed diabetes mellitus Diabetes 26/6 546 1977

4 Friedman H S Gomes J A Tardio A Levites R & Haft J I Appearance of atrial rhythm with absent p wave in long-standing atrial fibrillation Chest 66 172 1974

5 Froment R Touboul P Gallavardin L Porte J & Dufour R Signification de la reduction spontanee de fibrillations auriculaires anciennes Arch Mal Cœur 3 315 1976

6 Goldberg L M Bristow S D Parker B M & Ritzmann L W Paroxysmal atrial tachycardia with atrioventricular block Its frequent association with chronic pulmonary disease Circulation 21 499 1960

7 Grover D N Mathur V S Shrivastava S & Roy S B Electromechanical correlation of left atrial function after cardioversion Br Heart J 33 226 1971

8 Halpern M M Spontaneous sinus rhythm after sev eral years of persistent atrial fibrillation in rheumatic valvular disease Postgrad Med 20 564 1956

9 Hanson R & Tuna N Spontaneous conversion of chronic atrial fibrillation of twenty two years dura tion of sinus rhythm Minn Med 5 543 1971

0 Harrison D C Robinson M & Kleiger R E Role of hypoxia in digitalis toxicity Am J Med Sci 256 352 1968

:1 Holzmann M Exhaustion of atrial fibrillation in aneurysmatic dilatation of the left atrium Cardiologia 48 92 1966

22 — Basic mechanism of atrial fibrillation In Sym posium on Cardiac Arrythmias Elsinore Denmark p 92 Astra Sodertalje 1970

23 Hurst J W & Logue R B The heart McGraw Hill New York 1974

24 Ikram H Nixon P G & Arcan T Left atrial function after electrical conversion to sinus rhythm Br Heart J 30 80 1968

25 Jose A D Effect of combined sympathetic and para-sympathetic blockade on heart rate and cardiac function in man Am J Cardiol 18 476 1966

26 Khan A H Haider R Boughner D R Oakley C M & Goodwin J F Sinus rhythm with absent P waves in advanced rheumatic heart disease Am J Cardiol 32 93 1973

27 Lewis J K Auricular fibrillation for many years with spontaneous reversion to sinus rhythm Stanford Med Bull 13 131 1955

28 Lloyd Mostyn R H Defective innervation of heart in diabetic autonomic neuropathy Br Med J 3 15 1975

29 Lysholm M E Nylin G & Quarnå K Relation between heart volume and stroke volume under physiological and pathological conditions Acta Radiol 15 237 1934

30 Peter R H Morris J J & McIntosh H D Rela tionship of fibrillatory waves and P waves in the elec trocardiogram Circulation 33 599 1966

31 Prinzmetal M Corday E Brill I C Oblath R W & Kruger H E The auricular arrhythmias Thomas Springfield 1952

32 Reeve R Galbraith G T Reeve F J S & Lin T K Sinus rhythm after prolonged atrial fibrillation complicated by sinus arrest and syncope Am Heart J 90 127 1975

33 Rowlands D J Logan W F W E & Howitt G Atrial function after cardioversion Am Heart J 74 149 1967

34 Saint Pierre A Alabouvette G & Perrin A Reg ularisation spontanee et tardive de fibrillations au riculaires permanentes Lyon Méd 227 7 627 1972

35 Schamroth L Coarse and fine atrial fibrillation method of evaluation relation to mechanism and aetiology of fibrillation In Symposium on Cardiac Arrhythmias Elsinore Denmark p 52 Astra Soder talje 1970

36 Seneviratne B I B Diabetic cardiomyopathy the preclinical phase Br Med J 1 1444 1977

37 Sodermark T Jonsson B Olsson A Oro L Wallin H Edhag O Sjogren A Danielsson M & Rosenhamer G Effect of quinidine on maintaining sinus rhythm after conversion of atrial fibrillation or flutter A multicenter study from Stockholm Br Heart J 37 486 1975

38 Storstein O & Rasmussen K Digitalis and atrial tachycardia with block Br Heart J 36 171 1974

39 Szekely P Sideris D A & Batson G A Mainte nance of sinus rhythm after atrial defibrillation Br Heart J 32 741 1970

40 Taguchi J T & Ryan J M Spontaneous conver sion of established atrial fibrillation Arch Intern Med 124 468 1969

41 Thurmann M & Janney J G The diagnostic importance of fibrillatory wave size Circulation 25 991 1962
42 Vaisrub S Spontaneous reversion to normal sinus rhythm in case of auricular fibrillation of long standing Can Med Assoc J 63 599 1950
43 Wheeler T & Watkins P J Cardiac denervation in diabetes Br Med J 4 584 1973
44 Zimmerman T J Basta L L & January L E Spontaneous return of sinus rhythm in older patients with chronic atrial fibrillation and rheumatic mitral valve disease Am Heart J 86 676 1973

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# Plasma Free Fatty Acids and the Incidence of Arrhythmias in Acute Myocardial Infarction during Treatment with Small Doses of Subcutaneous Heparin or Warfarin

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ABSTRACT In a prospective trial 99 patients with a history of AMI of less than 12 hours were allocated at random to treatment with subcutaneous heparin 5000 IU twice daily, (51 patients) or warfarin (48 patients) In a subsample of 21 patients 11 in the warfarin group and 10 in the heparin group fasting FFA analyses were performed before and 2 hours after administration of anticoagulants on days 1 and 2 No measurable increase in FFA concentrations was demonstrated in the heparin treated patients in spite of a significant influence on the thrombin clotting time The frequency of ventricular arrhythmias as detected by continuous tape recordings was equal in the two groups It is concluded that subcutaneous heparin 5000 IU every 12 hours can be administered to patients with AMI without increasing the risk of arrhythmias as compared with warfarin

*Key words* free fatty acids arrhythmias acute myocardial infarction subcutaneous heparin

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In a prospective randomized double blind trial Warlow et al (18) administered small doses of heparin (5000 IU every 12 hours) subcutaneously to patients with acute myocardial infarction (AMI) They found that administration of heparin initiated within 12 hours from the onset of AMI reduced the frequency of deep venous thrombosis (DVT) diagnosed by the [125]I fibrinogen technique from 17% in the control group to 3% in the heparin group Such small doses of heparin do not give haemorrhagic complications in patients with AMI and no laboratory control is needed Warlow et al noted however that 8 of 63 patients in their heparin group developed ventricular tachycardia whereas only 4 of 64 patients in their control group did so This difference ($p>0.3$) could have occurred by chance

Oliver et al (14) found a positive correlation between high levels of serum free fatty acids (FFA) and the incidence of ventricular arrhythmias (and deaths) among 200 patients with AMI It has further been shown that therapeutic doses of i v heparin give a significant rise in FFA levels in patients with AMI (12 15) The former authors (12) however did not find any correlation between the FFA levels and the incidence of ventricular arrhythmias

The postheparin rise in FFA is thought to be brought about by activation of lipoprotein lipase Patients with higher initial triglyceride concentrations seem to have a greater increase in FFA levels after heparin (15)

Finally it has been shown that even small amounts of i v heparin (1–10 IU/kg) are accompanied by a rise in lipoprotein lipase activity (7 11)

The present study was undertaken in order to evaluate the influence of small doses of subcutaneous heparin on FFA levels and on the incidence of arrhythmias in patients with AMI Control patients selected by random allocation were given warfarin instead of heparin

## PATIENTS AND METHODS

The study comprised patients admitted to the Medical Intensive Care Unit in Medical Department 8 Ullevål Hospital with a history of AMI of less than 12 hours Patients with cardiogenic shock or already subjected to resuscitation or treated with anticoagulants or with contraindications to anticoagulant therapy (ongoing bleeding or known bleeding tendency) were excluded

When admitted to the trial the patients were allocated to treatment groups (warfarin or heparin) by the use of sealed envelopes preformed by our statistician on the basis of random numbers

*Abbreviations* AMI=acute myocardial infarction DVT=deep venous thrombosis FFA=free fatty acids

Table 1 *Some clinical data on patients in the two treatment groups*

| | Heparin | Warfarin |
|---|---|---|
| No of pats | 51 | 48 |
| Females males | 19 32 | 16 32 |
| Mean age (y) | 67 3 | 65 8 |
| *Previous history (no of pats)* | | |
| Angina pectoris | 32 | 24 |
| Heart infarction | 14 | 14 |
| Heart failure | 10 | 7 |
| Hypertension | 17 | 18 |
| Apoplexia cerebri | 3 | 2 |
| Claudicatio intermittens | 2 | 1 |
| Diabetes mellitus | 3 | 0 |
| *Medication prior to the present illness (no of pats)* | | |
| Digitalis | 15 | 12 |
| Diuretics | 8 | 9 |
| β blockers | 6 | 8 |
| Antihypertensives (others) | 13 | 14 |
| *Duration of symptoms until inclusion (no of pats)* | | |
| 0–3 h | 15 | 15 |
| 4–6 h | 18 | 12 |
| 7–12 h | 18 | 21 |
| *Symptoms and signs on admission (no of pats)* | | |
| Coronary pains | 45 | 40 |
| Hypotension | 4 | 3 |
| Left heart failure | 19 | 18 |
| Pulmonary oedema | 0 | 5 |
| *Size of infarction (no of pats)* | | |
| Small | 13 | 14 |
| Intermediate | 30 | 26 |
| Large | 8 | 8 |
| Maximum mean value of ASAT (U/l) | 208 | 222 |

Heparin (Nyegaard Oslo Norway) was given subcutaneously 5000 IU every 12 hours generally for 7 days Uncomplicated cases were routinely mobilized 3–4 days after the acute onset In complicated cases with longer confinement to bed heparin prophylaxis was prolonged

Warfarin (Marevan Nyegaard Oslo Norway) was given in standard doses on the first 2 days thereafter in doses aiming at Thrombotest values between 5 and 12% Warfarin was routinely continued until discharge from hospital

Heart rhythm was continuously registered by a tape recorder (Holter Avionics Research Products Corp ) from immediately after inclusion into the study and for 24 hours The recordings were analysed by two of us independently In addition a continuous cardioscopic monitoring with registration of any arrhythmia observed was undertaken for 72 hours

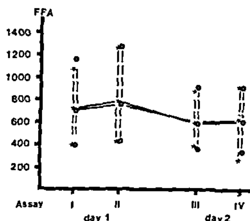



*Fig 1* Mean values and distribution of plasma FFA levels (μEq/l) in 21 patients with AMI 10 treated with heparin (★) and 11 with warfarin (O) Normal range 300–800 μEq/l

Fasting blood samples were drawn before and 2 hours after administration of anticoagulants in the first and second morning after admission FFA analyses were performed according to Dole (4) with the modification of Trout (16) EDTA blood was kept on ice water until centrifugation and the plasma was frozen until analysis Fasting triglycerides were measured in the first and second morning

Clinical registration—with special reference to heart failure shock bleeding or thromboembolic complications—was performed during hospital stay usually for 2 weeks

The size of infarction was estimated by evaluation of ASAT and ECG recordings and classified as follows Small infarction non transmural infarction and a maximum value of ASAT below 120 U/l (upper normal limit 40 U/l) Large infarction electrocardiographic extensive anterior infarction or anterior and inferior infarction and a maximum value of ASAT above 350 U/l The remaining infarctions were classified as intermediate

## RESULTS

A total of 140 envelopes were preformed and all of them were drawn One patient was later excluded because cardiac resuscitation had been performed before admission that is according to the reasons listed for exclusion Forty patients were later excluded because the diagnosis of AMI was not confirmed Thus 99 patients were included in the study 51 treated with heparin and 48 with warfarin The distribution between the two groups by age and sex size of infarction previous history medication prior to the present illness duration of symptoms until inclusion into the study and symptoms and

Table II *Arrhythmias detected during the first 24 hours after admission by the Holter recording system or by conventional monitoring in 99 patients with AMI treated with heparin or warfarin*

VES=ventricular extrasystoles VT=ventricular tachycardia HR=heart rate

| | Holter recording system | | Conventional monitoring | |
|---|---|---|---|---|
| | Heparin ($n$=31) | Warfarin ($n$=36) | Heparin ($n$=20) | Warfarin ($n$=12) |
| Warning ventricular arrhythmias | 25 | 31 | 8 | 4 |
| Frequent VES (>5/min) | 16 | 22 | 2 | 3 |
| Paired VES | 14 | 19 | 6 | 3 |
| Multifocal VES | 15 | 23 | 4 | 2 |
| Short VT (HR>120) | 11 | 15 | 3 | 1 |
| Ventricular fibrillation | | 1 | 1 | |
| Ventricular accelerated rhythm (HR 55–120) | 6 | 12 | | |
| Intermittent atrial fibrillation | 7 | 4 | | |
| Supraventricular tachycardia (HR>120) | 9 | 8 | 1 | |
| Sinoatrial block | 2 | 3 | 2 | 1 |
| Atrioventricular block 2nd–3rd degree | 3 | 2 | 1 | 1 |

signs on admission is given in Table I It is obvious that the comparability of the two groups is good

*FFA analyses* Blood samples from a subsample of 32 patients consecutively entering the trial were analysed Eleven patients were later excluded because of non verified diagnosis of AMI Thus the following results refer to 21 patients with a verified diagnosis of AMI 11 in the warfarin and 10 in the heparin group

As shown in Fig 1 the mean values of FFA were similar in the two treatment groups before and after administration of anticoagulants As a mean the values were within the upper normal limit Thus the subcutaneous administration of 5 000 IU heparin did not give any measurable increase in FFA

The mean fasting triglycerides were 1 89 and 1 60 mmol/l in the heparin group and 1 75 and 1 80 mmol/l in the warfarin group on the first and second day respectively

*Arrhythmias* Of the 99 patients with AMI 67 had tape recordings technically sufficient for evaluation 31 in the heparin group and 36 in the warfarin group The frequency of various arrhythmias is listed in Table II The frequency of ventricular and other arrhythmias is similar in the two groups

The results from the cardioscopic registration of the 32 patients (20 heparin and 12 warfarin treated) whose tape recordings were not satisfying are also listed in Table II A total of 71 so-called warning ventricular arrhythmias were registered in 33 patients in the heparin group compared to 88 such registrations in 34 patients in the warfarin group It appears that more than one type of ventricular arrhythmia may have been listed in one and the same patient

The results from the cardioscopic registrations performed 24–72 hours after admission are listed in

Table III *Arrhythmias detected 24–72 hours after admission by conventional monitoring in 99 patients with AMI treated with heparin or warfarin*

Abbreviations as in Table II

| | Heparin ($n$=51) | Warfarin ($n$=47) |
|---|---|---|
| Warning ventricular arrhythmias | 10 | 8 |
| Frequent VES | 6 | 3 |
| Paired VES | 8 | 3 |
| Multifocal VES | 6 | 6 |
| Short VT | 2 | 3 |
| Ventricular fibrillation | 1 | 3 |
| Ventricular accelerated rhythm | | 1 |
| Intermittent atrial fibrillation | 11 | 10 |
| Supraventricular tachycardia | 2 | 3 |
| Sinoatrial block | | 3 |
| Atrioventricular block 2nd–3rd degree | 4 | 4 |

Table IV *Distribution of complications and specific treatment given during hospitalization in the two treatment groups*

| | No of pats | |
|---|---|---|
| | Heparin group | Warfarin group |
| Total | 51 | 48 |
| Coronary pains | 33 | 36 |
| Hypotension | 5 | 5 |
| Cardiogenic shock | 3 | 3 |
| Left heart failure | 30 | 34 |
| Pulmonary oedema | 1 | 1 |
| Reinfarction | 3 | 1 |
| Venous thromboemboli | | 1 |
| Arterial emboli | 2 | 2 |
| Deaths | 7 | 6 |
| *Specific treatment given* | | |
| Digitalis | 35 | 40 |
| Diuretics | 32 | 33 |
| Morphine | 47 | 47 |
| Xylocaine | 15 | 21 |
| Procainamide | 2 | 3 |
| Verapamil | 6 | 7 |
| β blockers | 14 | 18 |
| Atropine | 2 | 3 |
| Disopyramide | 1 | 1 |
| Electroshock | 3 | 3 |
| Intracardial pacemaker | 5 | 4 |

Table III No difference is seen between the 51 patients in the heparin group (totally 22 registrations of warning ventricular arrhythmias in 10 patients) and the 47 patients (one patient died before 72 hours) in the warfarin group (totally 15 registrations of warning ventricular arrhythmias in 8 patients)

*Complications (Table IV)* No clinically important bleedings were recorded during the study One patient in the warfarin group developed pulmonary embolism In the heparin group 2 patients experienced arterial emboli one of them cerebral with blindness the other in an artery of the leg In the warfarin group there were also 2 patients with arterial emboli both of them cerebral

A total of 7 deaths occurred in the heparin group and 6 in the warfarin group and all of them were subjected to autopsy All deaths were related to cardiac emergencies such as ventricular fibrillation heart rupture and cardiogenic shock

As regards the frequency of special medication given during hospitalization no differences were noted between the two groups

No attempts were made for a more detailed diagnosis of DVT for instance by the $^{125}$I fibrinogen technique

## DISCUSSION

Patients with AMI are threatened by thromboembolic complications reflected by a high incidence of hypercoagulability of their blood (1 5) The frequency of DVT after an AMI has been found to be 17–38%, using the $^{125}$I fibrinogen method in patients not anticoagulated (9 10 13 18) Anticoagulation with full doses of heparin or warfarin has been shown to significantly reduce this frequency (8 13 19 20) Based on a review of clinical trials on treatment with anticoagulants of patients with AMI Chalmers et al (3) recently concluded that evidence favoured their use in the hospital phase of AMI In the randomized controlled trials in their survey the incidence of clinically detected thromboembolic events were reduced from about 21% in the untreated groups to about 11% in the anticoagulated ones

With the earlier mobilization of AMI patients in recent years the frequency of clinically detected thromboembolic complications is probably decreasing (2) However Maurer et al (9) showed that more than 50% of $^{125}$I fibrinogen thromboses developed during the first 72 hours after the onset of AMI and more than 70% during the first 5 days Furthermore Arnesen et al (1) found that about 60% of patients with AMI had laboratory evidence of fibrin formation in their blood on day 4 These findings still seem to support the use of anticoagulants for the prevention of venous thromboembolic complications during the acute phase of AMI

Full anticoagulation bears a considerable risk of bleeding and requires careful control An adequate prophylactic effect of small doses of subcutaneous heparin as regards the frequency of $^{125}$I fibrinogen thromboses in AMI patients has recently been described (6 18) This prophylaxis can be given with immediate effect avoidance of bleeding complications and without need of laboratory control

The frequency of ventricular tachycardia noted by Warlow et al (18) in patients with AMI receiving small doses of subcutaneous heparin prompted us to perform this study Because of the possible mechanism of heparin induced activation of lipoprotein lipase with consequent increase in plasma FFA which have been found to be arrhythmogenic in patients with AMI (7 12 14 15) assays of FFA

were performed on a randomized subsample of patients

In our study however we did not find any measurable increase in FFA concentrations after 5000 IU heparin given subcutaneously every 12 hours Nevertheless a significant influence on a sensitive coagulation test the thrombin clotting time was noted (1) Ham and Slack (7) found activation of lipoprotein lipase by heparin in concentrations down to about 0 025 IU/ml plasma when given i v The subcutaneous doses of 5000 IU given 12 hourly corresponding to plasma concentrations of up to 0 04 IU/ml (1) are probably too small to give any detectable increase in FFA levels in the circulation

With respect to the frequency of ventricular arrhythmias detected on continuous tape recordings we did not find any difference between the two treatment groups Thus the difference noted by Warlow et al (18) could not be confirmed The total frequency of arrhythmias registered in the present study corresponds well with that reported in similar studies (17)

In conclusion subcutaneous heparin 5000 IU every 12 hours can be given to patients with AMI without increasing the risk of arrhythmias as compared with warfarin

## REFERENCES


1 Arnesen H Bjerkedal I Skjæggestad Ø & Godal H C Plasma free fatty acids and small doses of subcutaneous heparin in acute myocardial infarction Thromb Res 14 541 1979
2 Bassan M M & Rogel S Anticoagulants Changing and unchanging indications Cardiology Today 6 4 1978
3 Chalmers T C Matta R J Smith H Jr & Kunzler A M Evidence favoring the use of anticoagulants in the hospital phase of acute myocardial infarction N Engl J Med 297 1091 1977
4 Dole V P A relation between non-esterified fatty acids in plasma and the metabolism of glucose J Clin Invest 35 150 1956
5 Fulton R M & Duckett K Plasma fibrinogen and thromboemboli after myocardial infarction Lancet 2 1161 1976
6 Habersberger P G Pitt A & Andersen S T Venous thrombosis in myocardial infarction Comparison in heparin dosage Br Heart J 35 538 1973
7 Ham J M & Slack W W The effect of small doses of heparin on platelet adhesiveness and lipoprotein lipase activity before and after operation Br J Surg 55 227 1968
8 Handley A J Emerson P A & Fleming P R Heparin in the prevention of deep vein thrombosis after myocardial infarction Br Med J 2 436 1972
9 Maurer B J Wray R & Shillingford J P Frequency of venous thrombosis after myocardial infarction Lancet 2 1385 1971
10 Murray T S Lorimer A R Cox F C & Lawrie T D V Leg vein thrombosis following myocardial infarction Lancet 2 792 1970
11 *Negus D Pinto D J & Slack W W Effect of* small doses of heparin on platelet adhesiveness and lipoprotein lipase activity before and after surgery Lancet 1 1202 1971
12 Nelson P G Effect of heparin on serum free fatty acids plasma catecholamines and the incidence of arrhythmias following acute myocardial infarction Br Med J 3 735 1970
13 Nicolaides A N Kakkar V V Renney J T G Kidner P H Hutchison D C S & Clarke M B Myocardial infarction and deep-vein thrombosis Br Med J 1 432 1971
14 Oliver M F Kurien V A & Greenwood T W Relation between serum free fatty acids and arrhythmias and death after acute myocardial infarction Lancet 1 710 1968
15 Russo J V Friesinger G C Margolis S & Ross R S Heparin and ventricular arrhythmias after myocardial infarction Lancet 2 1271 1970
16 Trout D L Estes E H Jr & Friedberg S J Titration of free fatty acids of plasma A study of current methods and a new modification J Lipid Res 1 199 1960
17 Vetter N J & Julian D G Comparison of arrhythmia computer and conventional monitoring in coronary care unit Lancet 1 1151 1975
18 Warlow C Beattie A G Terry G Ogston D Kenmure A C F & Douglas A S A double blind trial of low doses of subcutaneous heparin in the prevention of deep-vein thrombosis after myocardial infarction Lancet 2 934 1973
19 Working Party on anticoagulant therapy in coronary thrombosis to the Medical Research Council Assessment of short term anticoagulant administration after cardiac infarction Br Med J 1 335 1969
20 Wray R Maurer B J & Shillingford J P Prophylactic anticoagulant therapy in the prevention of calf vein thrombosis after myocardial infarction N Engl J Med 288 815 1973

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# Influenza A1 Myocarditis in Conscripts

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ABSTRACT The incidence of viral myocarditis was studied prospectively at the Central Military Hospital 1 in Finland in connection with an A1 virus influenza epidemic in Jan 1978 Of 104 conscripts taken consecutively to hospital because of a sudden respiratory infection 41 had serologically confirmed influenza A, 37 were serologically negative, while in 26 it was not possible to carry out complete virological analyses Six of the serologically confirmed influenza patients had acute myocarditis on the basis of serial electrocardiographic ST segment and/or T wave changes unresponsive to $\beta$-blockade The incidence of the influenza A myocarditis was thus 9 % of the 67 verified and suspected cases of influenza taken together Multidirectional echocardiography revealed regional myocardial dysfunction in all the influenza patients with myocarditis, the MB CK isoenzyme was elevated in 3 of them The ECG changes found in connection with influenza thus commonly indicate the presence of myocardial involvement, usually a mild one

*Key words* myocarditis influenza echocardiography creatine kinase isoenzymes
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A virus infection is often accompanied by myocarditis However clinically asymptomatic myocarditis frequently remains undiagnosed because no ECG is recorded (20) The myocarditis caused by Coxsackie B virus is well known (14) Mild myocarditis is evidently not rare in conjunction with other viral diseases either As many as one third of the patients with influenza echo virus or Mycoplasma infections have been found to have ECG changes indicative of myocardial irritation (8)

In Jan 1978 an epidemic caused by the influenza A1 virus spread to Finland s garrisons The frequency of myocarditis in conjunction with this influenza was studied prospectively at the Central Military Hospital 1

## PATIENTS AND METHODS

All 104 conscripts admitted to the Central Military Hospital 1 during Jan 9–31 1978 because of a sudden influenza like infection were examined in accordance with a myocarditis programme The patients were either serving in Helsinki's garrisons or were on leave from other garrisons in the Helsinki area

An ECG was recorded on the 1st and 3rd days in hospital and 10–20 days thereafter Paired sera for virus antibody determinations were taken simultaneously with the 1st and 3rd ECGs Serum from each patient was frozen on admission for any other later enzyme determinations If ST segment or T wave changes or conduction disturbances arousing suspicion of myocarditis (9) were present the ECG was followed for several days In these patients at least two chest X rays were taken at one week intervals An attempt was made to exclude non specific and functional vasoregulatory ST T wave changes by their normalization following administration of propranolol (1 4) Those with possible myocarditis were subjected to a careful cardiological examination and multidirectional echocardiography (11) The serum samples from the myocarditis patients taken on admission were later analysed for aspartate aminotransferase and creatine kinase (CK) enzymes by routine methods The cardiospecific MB CK isoenzyme was determined electrophoretically (15) The influenza A and adenovirus antibody titres of the paired sera of all patients were studied in the Central Public Health Laboratory using the complement fixation technique Patients who were suspected of having myocarditis underwent extensive virus antibody screening Unless significant increases in the antibody titres were noted the influenza A antibodies were further studied with the haemagglutination inhibition technique

## RESULTS

Eight of the 104 patients had ECG patterns indicating acute myocarditis Echocardiography revealed that all myocarditis patients also had local myocardial contraction abnormalities The diagnosis of influenza A was confirmed serologically in 41 patients 6 of them had myocarditis However this incidence of 15 % may be somewhat too high since it was possible to carry out complete virological analyses for 78 of the 104 patients while all the

Table I *Relevant findings of myocarditis caused by influenza A1*

Borderline asynergy indicates slight abnormality of the cardiac precordial thrust
*LVEDV*=*left ventricular end-diastolic volume*

| Pat no | Clinical features | Electrocardiogram | | Echocardiography | | |
|---|---|---|---|---|---|---|
| | | *ST T changes* | *Duration* | *Asynergy* | *Site* | LVEDV (ml) |
| 1 | | T↓ V4–5 | 1 day | Hypokinesia | Low anteroseptal anterolateral | 164 |
| 2 | | T↓ II III aVF | 1 week | Hypokinesia | Low anteroseptal anterolateral | 125 |
| 3 | S3-gallop borderline asynergy | T↓ III aVF V4–6 | 2 weeks | Hypokinesia | Anterolateral apical | 174 |
| 4 | | T↓ III aVF | 1 week | Akinesia paradoxical | Low anteroseptal | 184 |
| 5 | Borderline asynergy | T↓ V3–4 | 1 day | Hypokinesia | Anteroseptal inferior | 155 |
| 6 | S3 gallop borderline asynergy | ST↑ I II III aVF V2–6<br>T↓ aVF V2–6 | 6 weeks | Akinesia paradoxical | Anteroseptal | 132 |

myocarditis patients were studied serologically No serological signs of influenza were found in 37 patients 2 of them had myocarditis of unknown aetiology Thus the true incidence of influenza myocarditis may be 9% (6 out of 67 patients) if the serologically verified cases of influenza (41 patients) and the suspected cases (26 patients) are taken together whereas patients with negative serology are eliminated The adenovirus antibody titre rose significantly in 9 patients 3 of them had a double infection since the influenza A antibody titre rose at the same time but not a single patient with a double infection was found to have myocarditis

The influenza A1 was clinically mild The body temperature was 38–39°C for 2–3 days and other local symptoms included headache sore throat and muscle pains

The relevant findings in the influenza myocarditis patients are given in Table I All 6 patients had changes in the very first ECG which was recorded 1–2 days after the onset of symptoms On the other hand none of the patients studied had subjective symptoms suggesting myocarditis such as chest pains dyspnoea or sensation of arrhythmia The findings at clinical examinations were scant 2 patients had ventricular gallop even in the sitting position 3 had a slightly asynergic cardiac thrust on palpation but none had a clear paradoxical cardiac pulsation Cardiac physical findings in 3 patients were completely normal No pericardial friction rubs were heard The chest X rays were normal ECG changes gradually normalized completely in 5 patients as soon as in 1–2 weeks but in one patient clearly the most severe case of myocarditis (no 6) they persisted for 6 weeks Later he also experienced chest pains and very frequent supraventricular premature beats induced by exercise

Echocardiography was performed in the second week of the disease because of an ECG pattern indicating myocarditis All 8 patients had clearly abnormal regional myocardial changes characteristic of myocarditis local loss or reduction of systolic wall motion and an altered myocardial echo line generation (12) Such abnormalities were localized in the ventricular regions indicated by the ECG changes

A rise in the MB CK isoenzyme was found in 3 subjects even though 2 of them had normal total CK values The serum ASAT was elevated in one patient only again no 6 with the most severe myocarditis

In 6 myocarditis patients the diagnosis of influenza was based on either at least a four fold rise in antibody titres found with the complement fixation technique or else H1N1 type influenza A antibodies as shown with the haemagglutination inhibition technique

## DISCUSSION

According to the weekly reports from the Virus Laboratory most of the viruses isolated during the

| B–CK | Notes |
|---|---|
| | Junctional slow rhythm |
| | Junctional slow rhythm |
| | Complicated by pneumococcal pneumonia |
| | Later chest pains and numerous premature beats during exercise |

study were influenza A1 type H1N1 Thus most of our influenza patients had evidently the USSR A1 type influenza which caused an epidemic in the Soviet Union in Dec 1977 (22) The infection among conscripts in Finland in Jan 1978 was quite mild

The high incidence of myocarditis found in this study is not surprising In fact our figure of about 10% confirms several previous observations (5 8 13 18 19) The diagnosis of subclinical viral myocarditis is essentially dependent on whether an ECG is recorded at the initial stage of the disease During the 1957 Asian influenza epidemic ECG changes were found in 14% of patients admitted to hospital (5) and even in as many as 75% (19) Histological changes indicative of myocarditis were seen in 10 of 33 patients who died within the first eight days from the inception of Asian influenza (13) During the 1972 influenza A2 epidemic in Britain 43% of the patients treated at home had ECG changes (18) These studies (18 19) did however take into account even extremely non specific ECG changes sinus bradycardia or sinus tachycardia alone or a mere flattening of the T wave in one or more leads

Influenza myocarditis was clearly significant clinically in only one of our patients In the others the myocarditis was quite subclinical and was discovered only by the systematic ECG recordings Lewes et al (8) noted myocarditis in patients who complained of muscle pains All our myocarditis patients admitted that they had some kind of muscle pains but so did the other influenza patients

The primary diagnosis of myocarditis is based on serial ECG changes The interpretation of ECGs in mild myocarditis is however uncertain Notably the ECG changes in 2 of our patients were present only during the space of 24 hours The ECG finding demonstrated a mild inflammatory process fairly locally too In only one patient did the changes indicate diffuse inflammation Most typically the T waves became inverted in the inferior and/or anterolateral precordial leads In mild myocarditis these changes gradually normalized within only 1–2 weeks A very important indication of the pathology of even these slight changes was their irreversibility with propranolol which in turn eradicates the vasoregulatory ST-T changes (1 4)

Another method of confirmation in our hands has been echocardiography which brought out the fact that the often quite mild and non specific ECG changes in these patients were not just insignificant fever reactions since a correspondence with these changes is also found in dysfunction of the mechanical contractility of the myocardium Multidirectional echocardiography was originally developed to estimate the regional contraction dysfunction in the left ventricular wall motions in acute myocardial infarction (11) In myocarditis the asynergia of the systolic wall motion appears as akinesia and even as a paradoxical motion sometimes only as hypokinesia (12) In addition changes take place in the myocardial echo line patterns on the basis of which the age of the process can be estimated After a follow up for 3 months significant myocardial changes by echocardiography were found in only one of five patients

The cardiac specific isoenzyme of CK MB CK is still the most sensitive enzymatic index of myocardial infarction (3) An inflamed cardiac muscle also may release various enzymes into the circulation especially during severe myocarditis Half of our influenza myocarditis patients had MB CK enzymes It is noteworthy that 2 of these patients had total CK concentrations which remained normal The same seems to be true in minor ischaemic muscle damage (10) In the enzyme diagnosis of myocarditis attention should evidently be focused particularly on determining MB CK at the very initial stage of the disease

Luckily most asymptomatic myocarditis damage is slight and even local and rapidly and compl

curable Some cases are, however diffuse and may result in a permanent congestive cardiomyopathy (2 7) Different viruses vary greatly in this respect In animal experiments Coxsackie A9 has been found to cause slight local and rapidly curable myocarditis while Coxsackie B3 caused a diffuse inflammation leaving permanent scar formation (21) The present influenza A1 epidemic mainly re sulted in rather slight and transient signs of myocarditis

A significant clinical problem involved in undiag nosed myocarditis is the possibility of sudden death especially during physical effort (6 16) In experimental animals physical strain during the in- fection has been found to increase the amounts of viruses which can be isolated from the myocardium (17) For this reason special care should be taken with regard to physical exertion during virus epidemics which are known to be frequently linked with myocarditis e g influenza epidemics

## REFERENCES


1 Behar S & Kariv L Effect of propranolol on nonspecific S-T segment and T wave changes Dif ferentiation on coronary from noncoronary ECG changes Chest 63 376 1973
2 Editorial Viruses and cardiomyopathy Br Med J 2 850 1977
3 Editorial MB creatine kinase Lancet 1 313 1978
4 Furberg C Adrenergic beta blockade and elec trocardiographical ST T changes Acta Med Scand 181 21 1967
5 Gibson T C Arnold J Craige E & Curnen G C Electrocardiographic studies in Asian influenza Am Heart J 57 661 1959
6 Koskenvuo K Sudden deaths among Finnish con scripts Br Med J 2 1413 1976
7 Levi G F Proto C Quadri A & Ratti S Cox sackie virus heart disease and cardiomyopathy Am Heart J 93 419 1977
8 Lewes D Rainford D J & Lane F W Symptom less myocarditis and myalgia in viral and mycoplasma pneumoniae infections Br Heart J 36 924 1974
9 Lipman B S Massie E & Kleiger R E Clinical scalar electrocardiography Year Book Medical Pub- lishers Chigaco 1972
10 Marmor A Alpan G Keidar S Grenadier E & Palant A The MB isoenzyme of creatine kinase as an indicator of severity of myocardial ischaemia Lancet 2 812 1978
11 Nieminen M S Applications of multidirectional echocardiography in myocardial infarction Thesis Helsinki 1977
12 Nieminen M S & Heikkila J Echocardiographic features of acute myocarditis 3rd Symposium on Echocardiology p 68 Rotterdam 1979
13 Oseasohn R H Adelson L & Kaji M Cli nicopathological study of 33 fatal cases of Asian influenza N Engl J Med 260 509 1959
14 Sainani G S Krompotic E & Slodki S J Adult heart disease due to the Coxsackie virus B infection Medicine 47 133 1968
15 Somer H & Konttinen A Demonstration of se rum creatine kinase isoenzymes by fluorescence technique Clin Chim Acta 40 133 1972
16 Stevens D J & Underwood Ground K E Occur rence and significance of myocarditis in trauma Aerospace Med 41 776 1970
17 Tilles J G Elson S H Shaka J A Abelman W H Lerner A M & Finland M Effects of exercise on Coxsackie A9 myocarditis in adult mice Proc Soc Exp Biol Med 117 777 1964
18 Verel D Warrach A Potter C Ware L C & Richards D F Observations on the A2 England influenza epidemic A clinicopathological study Am Heart J 92 290 1976
19 Walsh J Burch G E White A Mogabgab W & Dietlein L A study of the effects of type A (Asian strain) influenza on the cardiovascular system of man Ann Intern Med 49 502 1958
20 Wenger N K Myocarditis In The heart (ed J W Hurst) p 1529 McGraw Hill New York 1978
21 Wilson M F Miranda Q R Chason J L & Lerner M Residual pathologic changes following murine Coxsackie A and B myocarditis Am J Pathol 55 253 1969
22 Zhdanov V M Zakstelskaya L Ya Isachenko V L Reznik V L Andreyev V P Lvov D K Yakhno M A Braude N A Pysina T V & Podchernyaeva R Ya Return of epidemic A1 (H1N1) influenza virus Lancet 1 294 1978

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# Relation between Ventricular Arrhythmias and Psychological Profile

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ABSTRACT The association between psychological characteristics and ventricular arrhythmias was investigated in 150 men (50 with manifest IHD, 50 with risk indicators of IHD and 50 healthy men) Arrhythmias were recorded with 24 hour Holter monitoring Psychological characteristics were assessed by the Emotions Profile Index and the Structured Interview for pattern A behaviour A depressive emotional state was associated with prognostically severe ventricular arrhythmia in healthy men, but not in men with overt IHD or risk indicators of IHD When clinical characteristics and age were taken into account depressiveness was—among healthy men—the second most important factor after high age The results suggest that—in absence of IHD or other cardiovascular disease—a depressive emotional state may participate in the formation of ventricular arrhythmia

*Key words* ventricular arrhythmias psychological profile depression ischemic heart disease

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Ventricular arrhythmias have been found to be associated with an increased risk of sudden death in patients with ischemic heart disease (IHD) (10 14 28) In these patients ventricular arrhythmias have been shown to increase with increasing age increasing severity of IHD and other clinical characteristics of advanced myocardial disease (19 25) Ventricular arrhythmias have also frequently been reported in patients with psychiatric disturbances (5 9 16 24) The association with clinical or subclinical signs of cardiac disease however remains unknown Systematic studies of the type of psychological derangement which may contribute to the formation of ventricular arrhythmias in patients with IHD or in healthy persons have not been presented

The aim of this study was to find out whether specific psychological traits or behaviour patterns exist in patients with ventricular arrhythmias The following questions were asked 1) Are there any associations between psychological characteristics as assessed by psychometric tests and ventricular arrhythmias? 2) Are such associations if demonstrated the same in patients with IHD patients who are at risk of developing IHD as judged by standard risk indicators and in healthy persons? 3) Are such associations if demonstrated dependent on other clinical characteristics known to be related to ventricular arrhythmias such as high age hypertension or cardiac enlargement?

## STUDY POPULATION

Three groups of 50 men each one with manifest IHD one with conventional risk indicators of IHD and one healthy control group were studied All 150 men were selected from a population of 4 000 men aged 40–65 employed in three large companies in the Stockholm area with well developed and efficient medical departments Regular health check-ups of all middle aged men including medical history and the assessment of coronary risk indicators were performed in these companies The subjects were selected in the following way

1) *The IHD group* all men registered in the medical departments as having myocardial infarction or angina pectoris Manifest IHD was found in 53 patients Two declined to participate Of the remaining 51 men 19 had angina pectoris only and 32 also had had myocardial infarction as verified by examination of hospital records The presence of angina pectoris was assessed by the London School of Hygiene questionnaire (8) In four patients typical angina pectoris was found both by questionnaire and clinical investigation A standardized exercise ECG with continuously increasing work load was performed in

*Abbreviations* IHD = ischemic heart disease VEBs = ventricular ectopic beats EPI = Emotions Profile Index

Table I *Distribution of risk indicators in each group*

| | IHD group | | Risk group | | Control group | |
|---|---|---|---|---|---|---|
| | *n* | % | *n* | % | *n* | % |
| Treatment for hypertension | 9 | 18 | 43 | 86 | | |
| Treatment for hyperlipidemia | 10 | 20 | 12 | 24 | | |
| Treatment for diabetes | 4 | 8 | 6 | 12 | | |
| *Smoking ≥20 cig /d* | 9 | 18 | 9 | 18 | 5 | 10 |

15 patients with less typical angina An ischemic ST segment depression of ≥1 mm was required for the diagnosis of IHD In 14 of these patients the presence of IHD was confirmed in one patient it could be ruled out by a repeated exercise test after i v administration of propranolol The mean age was 52 8 years at onset of IHD and 57 9 years at examination

2) *The risk group* 50 men who according to the health screening records *had one or more risk indicators of IHD* They were selected to match individually by age and occupation the men in the IHD group They were on treatment for hypertension hyperlipidemia diabetes or smoked 20 cigarettes or more per day The distribution of these characteristics in all groups is shown in Table I Atypical chest pain was reported by 21 subjects in the risk group who were all submitted to the standardized exercise ECG In 15 cases no ST changes were found whereas six cases showed atypical ST changes which were judged by two independent cardiologists not to be caused by ischemia

3) *The control group* 50 men free from IHD or known *risk indicators of IHD were selected from the company* pay rolls They matched individually by age and occupation the men in both the IHD and the risk group

Four of the 50 originally designated men in each of the risk and control groups did not want to participate They were replaced by four other men who were matched in the same way

## METHODS

All men were subjected to a continuous 24-hour ECG recording during normal work and home life An apical sternal lead was applied in which an exploring electrode was placed over the apex an indifferent one over the midsternum and a ground electrode on the right side of the chest (3) The Holter Avionics Electrocardiocorder Model 400 and Avionics Composite Electrocardiscanner Model 650 were used The same thoroughly trained laboratory technician analysed all tapes All abnormalities were written out on *ECG paper and reviewed by one of* the authors and by an independent cardiologist The validity of this technique has been discussed in detail by Hinkle et al (11)

All men were in sinus rhythm at the beginning of the recording The following ECG criteria were required for the identification of ventricular ectopic beats (VEBs) 1) Configuration different from regular QRS complex 2) Wide complex (~0 12 sec) 3) No preceding P wave 4) Prematurity and compensatory pause

In order to correlate psychological and clinical characteristics to ventricular irritability an index of ventricular ectopic activity based on the current prognostic classification by Lown et al (13) was formed Grade 1 = no VEBs during the recording Grade 2 = single uniform VEBs only Grade 3 = complex ventricular arrhythmia presence of couplets multiform VEBs *R-on T phenomenon or ventricular tachycardia*

In addition all men were subjected to the following examination procedures 1) A standard physical examination of the heart lungs and peripheral vessels 2) Systolic (phase 1) and diastolic (phase 5) blood pressure was measured in the supine position before and after 15 min rest *and in the sitting position A mean of the three measure*ments was calculated 3) Heart size was estimated from a frontal and a sagittal X ray of the chest Cardiac enlargement was defined as a relative heart volume of more than 500 ml (degree 2) borderline enlargement as 451–500 (de*gree 1)/m² BSA 4) An index (7) of past and present* smoking including number of cigarettes cigar and pipe smoking was applied 5) Average alcohol consumption during the past ten years was estimated and expressed in grams of absolute alcohol per week 6) Medication (drug and dosage) during the past month was recorded

Continuous psychological variables were investigated in a subsample of *90 randomly selected men 30 from each* group One man was excluded because of missing data The following psychological measures were used

A *The Emotions Profile Index* (EPI) (22) This is a brief paper and pencil test which measures a variety of emotional states It is based on a general theory of emotions developed by Plutchik and Kellerman (21) This theory suggests that personality traits are formed from an interplay between eight basic emotions In the EPI scale scores assessing the relative importance of these basic emotions in a person s life are obtained Aggressive (reflecting anger) an important feature of need for achievement and attainment Controlled (reflecting expectation or planfulness) Depressed (reflecting sadness) Distrustful (reflecting feelings of disgust or rejection) highly related to aggression *Dyscontrolled (reflecting impulsive*ness and a need for new experiences) Gregarious (reflecting joy) associated with affiliation Timid (reflecting an emotional state of fear) Trustful (reflecting an emotional state of acceptance)

The test–retest reliability measure of the EPI has yielded product–moment correlations for the different scales all over 0 90 (12) The validity of the EPI has also been demonstrated by obtaining significant correlations with a number of other personality measures including the Minnesota Multiphasic Personality Inventory and the Gough Adjective Check List

B *Behaviour pattern measure* Since a relationship between the type A behaviour pattern—characterized by impatience competitive striving and a sense of time ur

Table II *Ventricular arrhythmias in men with IHD men with risk indicators and healthy men*

| | IHD | | Risk | | Control | | Total | |
|---|---|---|---|---|---|---|---|---|
| | n | % | n | % | n | % | n | % |
| No VEBs | 8 | 16 | 17 | 34 | 19 | 38 | 44 | 29 |
| Single uniform VEBs | 19 | 38 | 13 | 26 | 16 | 32 | 48 | 32 |
| Complex ventricular arrhythmia (multiform VEBs coupled VEBs R-on T or VT) | 23 | 46 | 20 | 40 | 15 | 30 | 58 | 39 |

gency—and IHD has been reported (26) we were interested in examining the relationship between this behaviour pattern and ventricular arrhythmias All subjects were interviewed using the Structured Interview which was originally developed by Rosenman and Friedman (27) and judged to be the best assessment of pattern A behaviour

*Statistical methods*

Differences in arrhythmias between groups were analysed by means of the $\chi^2$ test When the number in one of the cells was below ten Fisher s exact probability test was used (2) In order to analyse the relationship between ventricular arrhythmias on one hand and all psychological variables age systolic and diastolic BP cardiac enlargement smoking and alcohol consumption on the other stepwise multiple regression analyses were performed In this way the independent significance of each psychological and clinical variable could be related to the severity of ventricular ectopic activity (none single uniform or complex (VEBs) Significance levels were tested by analysis of variance

## RESULTS

The incidence of ventricular arrhythmias in men with IHD in men with risk indicators and in controls is shown in Table II Ventricular ectopic activity when all kinds were grouped together was significantly more common in the IHD group than in the risk group ($p < 0.05$ $\chi^2 = 4.32$) and the control group ($p < 0.05$ $\chi^2 = 6.14$) Complex ventricular arrhythmias were also most common in the IHD less common in the risk and least common in the control group although the differences did not reach statistical significance

Single correlation coefficients between ventricular ectopic activity and all examined variables are shown in Table III Significantly correlated with VEB index at the 1% level were high age and a high EPI depression score Also evident from this table is the high intercorrelation between all psychological variables a consequence of the structure of the tests In the multiple regression analysis the effects of such intercorrelations are taken into account and each factor is analysed for its independent contribution to the explained variance in arrhythmias

When stepwise multiple regression analyses were performed with only psychological test scores as independent variables (Table IV) a high depression score emerged as the single independently significant variable When each group was analysed separately this was found only in the control group Type A behaviour which has been shown in large scale prospective studies to increase the risk of developing IHD was not significantly related to severity of ventricular arrhythmia in this study Subsequently the clinical characteristics were also included in the analyses (Table IV) High age was the most important arrhythmia discriminator both in the total sample and in the risk and control groups separately A high depression score was the next most important discriminator in the total sample and in the control group but did not quite reach statistical significance in these analyses In the IHD group a high SBP was the best discriminator between arrhythmia grades In order to further analyse the relationship between arrhythmia grade and the depression scale a cross tabulation was performed in each group (Table V) Differences in mean depression scores between subjects with and without complex ventricular arrhythmia were almost zero in the IHD group considerable in the risk group and greatest in the control group thus confirming the results of the regression analysis Finally the frequency of medication with possible effects on arrhythmias was recorded in each group $\beta$ Blocking agents were equally common in the IHD (48%) and in the risk group (46%) Digitalis was more common in the IHD (20%) than in the risk group (2%) No subject in the control group was on this kind of therapy

Table III *Correlation matrix all variables (product–moment correlations)*

| | | $V_1$ | $V_2$ | $V_3$ | $V_4$ | $V_5$ | $V_6$ | $V_7$ | $V_8$ | $V_9$ |
|---|---|---|---|---|---|---|---|---|---|---|
| $V_1$ | VEB index | | | | | | | | | |
| $V_2$ | Age | 0 45*** | | | | | | | | |
| $V_3$ | Smoking | 0 07 | −0 14 | | | | | | | |
| $V_4$ | Alcohol | 0 12 | 0 11 | 0 10 | | | | | | |
| $V_5$ | SBP | 0 18 | 0 19 | 0 06 | 0 03 | | | | | |
| $V_6$ | Cardiac enlargement | 0 21 | 0 19 | −0 11 | 0 09 | 0 14 | | | | |
| $V_7$ | Trust | 0 04 | −0 01 | 0 04 | −0 17 | −0 06 | 0 16 | | | |
| $V_8$ | Dyscontrol | 0 01 | −0 00 | −0 16 | −0 03 | −0 04 | −0 11 | −0 20 | | |
| $V_9$ | Timidity | 0 00 | −0 06 | −0 03 | −0 08 | −0 01 | −0 09 | 0 06 | −0 66 ** | |
| $V_{10}$ | Depression | 0.25** | 0 13 | −0 00 | 0 25** | 0 11 | 0 06 | −0 31** | −0 45*** | 0 32*** |
| $V_{11}$ | Distrust | 0 18 | −0 05 | 0 16 | 0 00 | −0 07 | 0 08 | −0 27** | 0 07 | −0 55*** |
| $V_{12}$ | Control | 0 13 | −0 12 | 0 02 | 0 05 | 0 08 | −0 14 | −0 22 | −0 05 | 0 32*** |
| $V_{13}$ | Aggression | 0 15 | 0 11 | −0 06 | 0 17 | −0 00 | 0 01 | −0 61*** | 0 20 | −0 31** |
| $V_{14}$ | Gregariousness | 0 01 | 0 01 | 0 01 | −0 08 | 0 09 | 0 11 | 0 71*** | 0 15 | −0 33*** |
| $V_{15}$ | Pattern A behaviour | 0 00 | 0 13 | −0 02 | −0 18 | 0 08 | 0 14 | 0 00 | 0 10 | −0 25** |

**$p<0$ 01 ***$p<0$ 01 (Due to the large number of correlations a 1% level was required for statistical significance )

## DISCUSSION

Our study confirmed that men with manifest IHD and men at risk of developing IHD have both more frequent and more malignant ventricular ectopics than healthy controls This finding will be described and discussed in detail in another report on the same sample of men (19) Our findings also confirmed the well recognized increase in the frequency of ventricular arrhythmias with increasing age (19 25) Of other clinical characteristics hypertension and cardiac enlargement were related to ventricular ectopic activity

When the significance of psychological factors for ventricular arrhythmias was analysed in the total

Table IV *Multiple regression analyses (computation discontinued at an F level of <1 0)*

| Group | *n* | Variables entered on steps 1 and 2 | *F* level | Explained variance (%) |
|---|---|---|---|---|
| *Psychological variables with VEB index* | | | | |
| IHD | 30 | Type B behaviour | 2 54 n s | 8 |
| | | EPI timid | 2 27 n s | 8 |
| Risk | 30 | EPI controlled | 3 07 n s | 10 |
| Control | 29 | EPI depressed | 5 40 | 12 |
| | | Type A behaviour | 1 75 n s | 6 |
| Total | 89 | EPI depressed | 7 22 * | 6 |
| | | EPI gregarious | 1 69 n s | 2 |
| *All variables with VEB index* | | | | |
| IHD | 30 | SBP | 4 17* | 8 |
| | | Pattern B behaviour | 3 40 n s | 12 |
| Risk | 30 | High age | 7 50* | 23 |
| | | Cardiac enlargement | 2 82 n s | 8 |
| Control | 29 | High age | 16 69** | 39 |
| | | EPI depressed | 3 65 n s | 8 |
| Total | 89 | High age | 18 95* | 20 |
| | | EPI depressed | 3 68 n s | 4 |

n s = Not significant *$p<0$ 05 critical *F* level ($n \geq 30$) 4 17 ($n \geq 60$) 4 00 *$p<0$ 01 critical *F* level ($n \geq 30$) 7 56 ($n \geq 60$) 7 08

| ...10 | $V_{11}$ | $V_{12}$ | $V_{13}$ | $V_{14}$ |
|---|---|---|---|---|
| * | | | | |
| 0 37* * | | | | |
| 0 21 | −0 04 | | | |
| 0 42*** | 0 25** | −0 64*** | | |
| 0 46 ** | −0 14 | −0 13 | −0 51 ** | |
| 0 02 | 0 12 | −0 19 | 0 15 | 0 13 |

sample a high depression score emerged as an independent factor This relationship was maintained in the separate analysis of healthy men but not in men with overt IHD or risk indicators of IHD It is suggested that in younger persons without signs of IHD or other cardiovascular disease psychological factors such as depression may be of importance for ventricular arrhythmic activity This was also indicated in a recent report by Bigger et al (3) who described two cases of clinical depression with frequent cardiac arrhythmias but without signs of overt cardiac disease The patients responded to imipramine a tricyclic antidepressant by decreasing their cardiac irritability A beneficial antiarrhythmic effect of imipramine was suggested in spite of the well recognized cardiotoxicity of tricyclic antidepressants in high doses Caution is needed however before comparing clinical depression as judged by external observation of a patient s behaviour with a high depression score of a psychometric scale which represents the patient s own description of his emotional state

That various psychological disorders may be associated with changes in the cardiac rhythm and other changes in the ECG has long been recognized and described in a number of psychiatric reports Examples of such reports include those of Heyer et al (9) and Blom (5) who observed changes in heart rate and configuration of the ECG complex but made no notes of ectopic activity Regestein concludes in an extensive review of the literature (24) that there is no specific link between a given type of psychological derangement and cardiac arrhythmia

The problem has also been studied in the reverse i e psychological symptoms in arrhythmic patients an approach which is more similar to our investigation Thus Zauner (29) considered obvious psychic symptoms to be present in 21 of 25 patients with frequent extrasystoles and symptomatic depression was included among the disorders The literature also contains several case histories of well investigated extremes (8 18 20) as illustrated by an 11 year old boy (23) who developed ventricular tachycardias when impatient or frustrated Lown et al (14) reported on a patient in whom ventricular arrhythmias were related to higher nervous activity Their patient was taught meditation techniques which allowed him to control his arrhythmic episodes

In experimental studies the significance of the autonomous nervous system for arrhythmia formation has been convincingly demonstrated Lown et al (15) showed that the repetitive extrasystole threshold in dogs was reduced to one third of the normal by a psychologically stressful environment

As previously outlined by Biörck and Vendsalu (4) and recently discussed in greater detail by Abildskov (1) and Lown et al (16) both the autonomous nervous system and higher nervous centers seem to participate in the pathogeneses of ventricular arrhythmias Emotional states of depression and sadness certainly involve the higher central nervous system and probably also influence peripheral autonomic nervous activity As depressiveness was associated with severity of arrhythmias in our control group but not in the IHD

Table V *Depression scores (mean ± S D ) and arrhythmia grades in the IHD risk and control groups*

| Group | No complex arrhythmia | Complex arrhythmia |
|---|---|---|
| IHD | 26 9±19 9 (*n* =16) | 27 1±15 5 (*n* =14) |
| Risk | 21 6±16 4 (*n* =20) | 30 0±17.3 (*n* =10) |
| Control | 16 5±11 6 (*n* =20) | 28 3±18 4 (*n* =9) |
| Total | 21 3+16 3 (*n* =56) | 28 3±16 4 (*n* =33) |

*group* the disease itself could not possibly act as a confounder causing both arrhythmias and depression In contrast it is suggested that a depressive emotional state may participate in the formation of severe ventricular arrhythmias in absence of IHD or other cardiovascular disease Whether the prognostic significance of these arrhythmias is equivalent to that of arrhythmias seen in myocardial disease remains to be investigated in future examinations

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## REFERENCES

1 Abildskov J A The nervous system and cardiac arrhythmias Circulation (Suppl) III 116 1975
2 Armitage P Statistical methods in medical research Blackwell Scientific Publications Oxford 1973
3 Bigger J T Giarcina E G V Perel J M Kantor S J & Glassman A H Cardiac antiarrhythmic effect of imipramine hydrochloride N Engl J Med 296 206 1977
4 Biörck G & Vendsalu A Studies in functional heart disease I A survey of an electrocardiographic material Acta Med Scand 155 361 1956
5 Blom G E Review of electrocardiographic changes in emotional states With clinical note on electrocardiograms of 193 psychotic patients J Nerv Ment Dis 113 283 1951
6 Chung E K Principles of cardiac arrhytmias Williams & Wilkins Baltimore 1977
7 Dyer A R Stamler J Bergson D M & Lindberg H A Relationship of relative weight and body mass index to 14-year mortality in the Chicago Peoples Gas Company J Chron Dis 28 109 1975
8 Harvey W P & Levine S A Paroxysmal ventricular tachycardia due to emotion possible mechanisms of death from fright JAMA 150 479 1952
9 Heyer H E Winans H M & Plessinger V I Alterations in the form of the electrocardiogram in patients with mental disease Am J Med Sci 214 23 1947
10 Hinkle L E Carver S T & Argyros D C The prognostic significance of ventricular premature contractions in healthy people and in people with coronary heart disease Acta Cardiol (Suppl) 18 5 1974
11 Hinkle L E Meyer J Stevens M & Carver S T Tape recordings of the ECG of active men Limitations and advantages of the Holter Avionics instruments Circulation 36 752 1967
12 Kellerman H & Plutchik R Emotion-trait interrelations and the measurement of personality Psychol Rep 23 1107 1968
13 Lown B Calvert A F Armington R & Ryan M Monitoring for serious arrhythmias and high risk of sudden death Circulation (Suppl) III 189 1968
14 Lown B Temte J V Reich P Gaughan C Regestein Q & Hai H Basis for recurring ventricular fibrillation in the absence of coronary heart disease and its management N Engl J Med 294 623 1976
15 Lown B Verner R & Corbalan R Psychologic stress and threshold for repetitive ventricular response Science 182 834 1973
16 Lown B Verner R L & Rabinowitz S H Neural and psychological mechanisms and the problem of sudden cardiac death Am J Cardiol 39 890 1977
17 Lynch J J Paskewitz D A Gimbel K S & Thomas S A Psychological aspects of cardiac arrhythmia Am Heart J 93 645 1977
18 McClure C M Cardiac arrest through volition Calif Med 90 440 1959
19 Orth-Gomer K Ventricular arrhythmias and risk indicators of ischemic heart disease Acta Med Scand To be published
20 Pickering T G & Miller N E Learned voluntary control of heart rate and rhythm in two subjects with premature ventricular contractions Br Heart J 39 152 1977
21 Plutchik R The emotions facts theories and a new model Random House New York 1962
22 Plutchik R & Kellerman H Emotions Profile Index Western Psychological Services Los Angeles 1974
23 Rahe R H & Christ A E An unusual cardiac (ventricular) arrhythmia in a child Psychiatric and psychophysiologic aspects Psychosom Med 28 181 1966
24 Regestein Q Relationships between psychological factors and cardiac rhythm and electrical disturbances Compr Psychiatry 16 137 1975
25 Rehnqvist N Ventricular arrhythmias after an acute myocardial infarction Prognostic weight and natural history Eur J Cardiol 7 169 1978
26 Rosenman R H Brand J Jenkins C D Friedman M Straus R & Wurm M Coronary heart disease in the western collaborative group study Final follow up experience of 8½ years JAMA 233 872 1975
27 Rosenman R H Friedman M Straus R Wurm M Kositchek R Halm N & Werthessen N T A predictive study of coronary heart disease JAMA 189 15 1964
28 Ruberman W Weinblatt E Goldberg J D Frank C W & Shapiro S Ventricular premature beats and mortality after myocardial infarction N Engl J Med 297 750 1977
29 Zauner J Über die Rolle psychischer Faktoren bei Herzrhythmusstörungen Z Psychosom Med 10 267 1964

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# Prognostication in Acute Cerebrovascular Disease

*Subjective Assessment and Test of a Prognostic Score*

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ABSTRACT Subjective assessment of the short term outcome and functional state at discharge was made shortly after admission in 200 consecutive patients with acute cerebrovascular disease (CVD) treated in a stroke unit The assessments proved correct in 59% of the patients The accuracy of the predictions was not significantly better in patients with a correct preliminary diagnosis than in those with a false When a known prognostic score for prediction of hospital mortality was tested on 179 of the patients with cerebral haemorrhage or infarction, a correct trend was noted The score was best applicable in patients with serious symptoms and those with only minor deficit on admission A high sensitivity of the score was combined with a relatively low specificity A true comparison between the predictive value of the score and the quality of the subjective assessments was difficult as the latter in addition to prediction of mortality also included prediction of the patient's functional state at discharge The degree of neurological deficit rather than the type of cerebrovascular lesion seemed associated with the short term outcome Improvement of the quality of prognostic assessments in CVD is warranted

*Key words* acute cerebrovascular disease prognosis stroke unit

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There are three main aspects of all medical problems diagnosis therapy and prognosis By tradition diagnosis and therapy have dominated most clinical discussions Prognosis and prognostication though closely associated with the former aspects have often received less consideration In order to evaluate the effect of any new treatment, patient groups with comparable prognosis must be defined Apart from characterization of prognostic groups is the even more difficult task of assessing prognosis in the individual patient

Although several factors are known to influence the short term prognosis in acute cerebrovascular disease (CVD) only a few attempts have been made to create prognostic indices or scores for these patients (12 14 20 30) and only two of them have been clinically evaluated (13 28) The aim of the present study was to evaluate the accuracy of subjective prognostication in patients with acute CVD and to test an existing prognostic score

## PATIENTS AND METHODS

Serafimerlasarettet serves a population of about 120000 inhabitants in Greater Stockholm and about 300 CVD patients are admitted annually

Criteria for admission to the Stroke Unit 1) Transient ischaemic attacks (TIA) Patients with one or more episodes of a focal neurological deficit with a duration of less than 24 hours within the last month Attacks of vertigo or syncope without focal neurological deficit are not included 2) Progressive and manifest stroke Patients with acute onset of focal neurological deficit during the previous week and without preceding head injury

Patients fulfilling any of these criteria in the Casualty Department are transferred to the Non Intensive Stroke Unit on a non selective basis They are examined regularly during the hospital stay and laboratory and X ray examinations are performed according to a strict investigation schedule Clinical findings spinal fluid spectrophotometry (16) and brain scintigraphy are the base for diagnosis Criteria for diagnosis and routines of the Stroke Unit have been presented elsewhere (3)

Two hundred consecutive patients with CVD treated in the Stroke Unit were included in the present study Patients with subarachnoid haemorrhage were not included There were 117 women mean age 75 years and 83 men mean age 70 years

In the Casualty Department a special record for patients with suspected CVD was filled in by the examining doctor (2) The patients were re-examined shortly after arrival in the Stroke Unit usually on the day of admission On the basis of history and physical findings on admission a preliminary diagnosis and predicted functional group at

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*Abbreviations* CVD=cerebrovascular disease TIA=transient ischaemic attack FG=functional group

Table I *Score for neurological evaluation of patients with acute stroke (13)*

Total maximum 100 points

| Factor | Score |
|---|---|
| *Mentation* | |
| *Level of consciousness* | |
| Fully conscious | 8 |
| Somnolent | 6 |
| Pre-comatose | 4 |
| Comatose | 0 |
| *Orientation* | |
| Oriented×3 | 6 |
| Oriented×1–2 | 3 |
| Disoriented | 0 |
| *Speech* | |
| Normal | 23 |
| Disconnected phrases | 15 |
| Aphasia | 10 |
| Dumb | 0 |
| *Cranial nerves* | |
| No conjugate deviation | 6 |
| Conjugate deviation | 0 |
| *Central facial function* | |
| Intact | 3 |
| Palsy | 0 |
| *Motor strength (each limb separately)* | |
| Normal strength | 5 |
| Pareses | 2 |
| Paralysis | 0 |
| *Performance disability status scale* | |
| Normal | 28 |
| Moderate impairment | 21 |
| Considerable impairment | 14 |
| Severe impairment | 7 |
| No performance at all | 0 |
| *Reflex* | |
| Normal | 3 |
| Spastic | 1 |
| No reflexes | 0 |
| *Sensation* | |
| Normal | 3 |
| Sensory abnormality | 1 |
| No response to pain | 0 |

discharge were set up by at least one consulting physician and one junior doctor The predicted functional group was then compared to the real functional state of the patient at discharge The average length of the hospital stay was 21 days

The classification of functional groups (FG) was as follows FG1 The patient is able to walk without assistance GF2 The patient needs mechanical aid to walk FG3 The patient is unable to walk on his own FG4 The patient is confined to bed or wheelchair

A prognostic score (maximum 100 points) for patients with a first episode of acute CVD modified after Mathew et al (20) and used by Frithz and Werner (13) was calculated for all patients (Table I) The calculations were based on the physical findings registered at the first examination in the Stroke Unit A prognostic index (tree) for patients with a first time cerebral infarction including a combination of the above prognostic score and different physical findings was created by Frithz and Werner (13) by multivariate analyses (AID analysis and multiple regression analysis (27)) This index was also tested on our patients with cerebral infarction

Validity of the score as regards mortality was expressed by sensitivity specificity and predictive value as calculated by the following formulae (7)

$$\text{Sensitivity} = \frac{a}{a+b} \times 100\%$$

$$\text{Specificity} = \frac{d}{c+d} \times 100\%$$

$$\text{Predictive value} = \frac{a}{a+c} \times 100\%$$

where $a$=deceased predicted dead by the score (true positives) $b$=deceased not predicted dead by the score (false negatives) $c$=survivors predicted dead by the score (false positives) $d$=survivors predicted alive by the score (true negatives)

The $\chi^2$ test was used for testing the significance of differences of proportions Degrees of significance were tested at 5 1 and 0 1% levels

## RESULTS

### *Diagnosis and mortality*

Distribution of patients by age final diagnoses and hospital mortality are shown in Table II Cerebral infarction including cerebral thrombosis and embolism was the predominant diagnosis (77%) and only 10% of the patients had cerebral haemorrhage As expected the mortality rate was highest in the latter group The majority of the 21 patients with TIA were already without or had only minor symptoms when examined on the first day in the Stroke Unit The hospital mortality among the remainder is shown in Table III in relation to the FG on day 1

### *Application of a prognostic score*

The relation between the prognostic score as registered on the first day in the Stroke Unit and hospital mortality in our 179 patients with cerebral infarction and haemorrhage are presented in Fig 1 Mortality fell with increasing score All four patients with a

Table II *Distribution of the 200 patients by age diagnosis and hospital mortality*

| | Age (y) | | | | Total | | Mortality (%) |
|---|---|---|---|---|---|---|---|
| | 50-59 | 60-69 | 70-79 | ≥80 | n | % | |
| Cerebral haemorrhage | 1 | 6 | 9 | 4 | 20 | 10 | 45 |
| Cerebral thrombosis | 9 | 27 | 49 | 26 | 111 | 55 5 | 14 |
| Cerebral embolism | 1 | 11 | 12 | 19 | 43 | 21 5 | 23 |
| TIA | 2 | 5 | 11 | 3 | 21 | 10 5 | 0 |
| Acute ill-defined CVD | | 2 | 2 | 1 | 5 | 2.5 | 40 |
| Total | 13 | 51 | 83 | 53 | 200 | | |

score <15 died those with a score >30 had a mortality rate below 25% and among patients with a score >60 mortality was very low The FGs at discharge are shown in Fig 2 in relation to the different score levels The proportion of patients discharged in a favourable condition (FGs 1 and 2) rose with an increasing score There was no clear pattern among patients discharged in worse functional condition (FGs 3 and 4)

The predictive value of the score is shown in Fig 3 Regarding mortality it was almost 90% at a score level of 20 and 77% at a score level of ≤24 20 out of 26 patients with such a score died The sensitivity and specificity of the score are shown in Fig 4 It is apparent that a high sensitivity was combined with a comparatively low specificity and vice versa A sensitivity of 90% corresponds to a specificity of around 60% and a specificity of 90% to a sensitivity of 60%

*Test of a prognostic tree*

A predictor tree for patients with cerebral infarction (13) has been applied in Fig 5 and compared to our 154 patients with a corresponding diagnosis Hospital mortality was 17% which is comparable to 20% in the original index based on 225 patients There was no significant difference between the total mortality rates in the two patient series Our patients with a score ≤24 had a mortality of 65% compared to 87% in the index material ($p<0\ 05$) In patients with combined right and left hemidisorder the present mortality rate was lower ($p<0\ 01$) In our patients with a score ≥25 the mortality rate was higher ($p<0\ 01$) The index mortality rates in patients with and without conjugate deviation were lower than in ours in the latter group the difference was significant ($p<0\ 05$)

*Subjective assessment of prognosis*

The proportions of patients in different FGs on the first day in the Stroke Unit and at discharge are presented with the predicted functional groups in Table IV At discharge more than half of the patients could walk on their own (FGs 1-2) and only 17% were bedridden or confined to a wheelchair The proportions of patients predicted for FGs 1-3 were overestimated whereas the proportion of those predicted for FG4 and the number of de

Table III *Mortality in relation to functional group on the first day in the Stroke Unit (patients with TIA are excluded)*

| | Patients | | Mortality (%) |
|---|---|---|---|
| | n | % | |
| FG1 | 48 | 27 | 4 |
| FG2 | 11 | 6 | 0 |
| FG3 | 34 | 19 | 3 |
| FG4 | 86 | 48 | 40 |
| Total | 179 | 100 | |

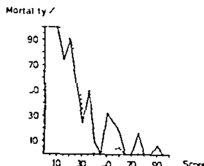



*Fig 1* Correlation between prognostic score and hospital mortality — = Frithz and Werner s patients (13)

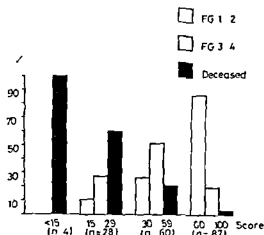



*Fig 2* Relationship between the prognostic score on ad mission and FG at discharge in 179 patients with cerebral infarction or haemorrhage

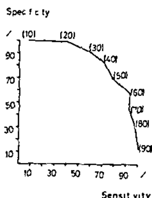



*Fig 4* Specificity as a function of sensitivity at different score levels (figures in parentheses)

ceased patients were underestimated The pre dicted hospital mortality rate was only 10% The subjective assessments in individual patients were poor Twenty of 26 seriously ill patients (prognostic score ≤24 on the first day in the Stroke Unit) died and in 12 (60%) of them we made correct predic tions

The predicted mortality rates were too low in all diagnostic groups Patients with a final diagnosis of cerebral haemorrhage had a mortality rate of 45% (predicted 30%) those with cerebral thrombosis 14% (predicted 7%) and those with cerebral emboli 23% (predicted 7%) The accuracy of the predic tions for the individual patients in different func tional groups is also shown in Table IV In all FGs except FG 1 86% correct predictions less than half of the individual prognostic assessments proved correct and the overall accuracy was 59%

In the 179 patients with cerebral infarction or haemorrhage a correct preliminary diagnosis had been made in 125 cases (70%) and a false in 54 cases (30%) In both patient categories the predicted proportions of patients in good functional state at discharge (FGs 1–2) were overestimated while the predicted mortality rates were underestimated Surprisingly the proportion of correct prognostic predictions was not significantly higher in patients with a confirmed preliminary diagnosis (61%) than in those with a false (54%)

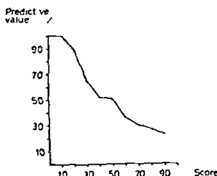



*Fig 3* Predictive value of the score

## DISCUSSION

Several factors are known to influence the prog nosis for the hospital period in patients with acute CVD e g advanced age impairment of conscious ness severe motor deficit conjugate ocular devia

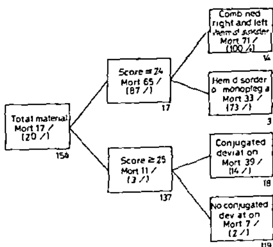



*Fig 5* Prognostic tree for patients with cerebral infarc tion Percentages in parentheses indicate mortality rates reported by Frithz and Werner (13)

Table IV *Percentage of real and predicted FGs of 179 patients with acute stroke*

| | FG1 | FG2 | FG3 | FG4 | Deceased |
|---|---|---|---|---|---|
| On day 1 | 27 | 6 | 19 | 48 | |
| At discharge | 36 | 15 | 11 | 17 | 21 |
| Predicted for discharge | 43 | 19 | 16 | 12 | 10 |
| Correct individual predictions | 86 | 48 | 45 | 37 | 43 |

tion bilateral plantar response and underlying medical complications on admission (6 18 22 24 29) However only a few attempts have been made to create prognostic groups or profiles by use of combinations of these single factors (13 28) Mathew et al (20) constructed a subjective prognostic score but they did not evaluate its predictive accuracy Frithz and Werner (13) who used a modification of the score constructed by Mathew et al (Table I) prognosticated retrospectively the outcome in 344 patients all below 70 years of age with a first time cerebrovascular accident An unusually high proportion (26%) of these patients had cerebral haemorrhages Death or survival was correctly predicted by the score in around 85% of the patients irrespective of a final diagnosis of cerebral infarction or haemorrhage

In hospitalized patients with stroke without diagnostic selection the mortality in the acute phase (during the first 3 or 4 weeks) ranges between 30 and 60% (4 17 19 21) Compared to these figures 19% mortality among our patients was low In hospital series with diagnostic selection the mortality in acute phase has been cerebral haemorrhage 55–98% (ours 43%) cerebral thrombosis 20–39% (ours 14%) and embolic brain infarction 25–67% (ours 23%) (10 11 15 26) Of course it must be kept in mind that the reliability of diagnosis of type of stroke is not high in general (1)

Although our patients had a higher mean age (73 years) than those of Frithz and Werner and 32% of them had had previous CVD a trial of the above prognostic score was considered of value especially if the result could be compared to that of a subjective assessment of prognosis When evaluating the score we noted a mortality rate of 100% in our few patients with a score below 15 but of less than 25% in those with a score above 30 These findings are in accordance with those of the previous authors In disagreement however we registered a definite mortality in patients with a high score (>65) Increasing score on the first day in the Stroke Unit was associated both with lower mortality rates and better functional state at discharge By use of a score level of ≤24 a predictive value of almost 80% was achieved A high sensitivity of the score was combined with a relatively low specificity

As the mortality trends of the tested prognostic score were in accordance with those of our patients a further test of the prognostic tree (index) (13) for patients with cerebral infarction and different prognostic profiles was considered valid (Fig 5) our patients with cerebral haemorrhage were too few to be tested correspondingly There was no significant difference between the total hospital mortality rates in the two patient series with cerebral infarction but in the subgroups our patients with a very low score (≤24) tended to have lower mortality rates than the index patient group while those with a higher score (≥25) had significantly increased mortality rates Differences in the characteristics of the two patient series e g the age distribution selection of patients and diagnostic procedures may account for these findings In its present form the prognostic index did not seem suitable for prognostication in subgroups of our unselected patients with cerebral infarction

The subjective prognostic assessments made on the first day in the Stroke Unit were generally too optimistic Overall functional groups and mortality were correctly predicted in almost 60% of the patients The accuracy of the predictions was not significantly better in patients with a correct preliminary diagnosis (cerebral infarction or haemorrhage) This finding suggests that the clinical state of the patient is more important for the immediate prognosis than the nature of cerebral damage and is in accordance with results of other authors (13)

It is difficult to make a true comparison between the predictive value of the prognostic score and the quality of subjective assessment The score was constructed with regard to prediction of mortality while the subjective prognostication in addition to that included prediction of the functional state of the patients discharged alive However the predictive value of the score was fair in patients with serious symptoms on admission to the Stroke Unit as well as in those with only minor neurological deficits The correctness of the subjective assess

ments was acceptable only in patients with minor symptoms on admission

To make better prognostic assessments other significant factors have to be considered Only neurological signs were evaluated in the tested score The subjective evaluation also included in directly other clinical factors e g cardiac disease which is a very common feature in stroke patients (18 23) Several reports have stated that heart dis ease either unspecified (5 8) or congestive (9 25) is associated with an impaired short term prognosis after stroke It is possible that consideration of such and other factors may help to improve the quality of prognostication during and after an acute stroke

## ACKNOWLEDGEMENTS

This study was supported by grants from the Swedish Planning and Rationalization Institute of the Health and Social Services (SPRI) and Axel Ax son Johnson Foun dation

## REFERENCES

1 Aho K Incidence profile and early prognosis of stroke Academic dissertation Helsinki 1975
2 von Arbin M Britton M de Faire U Helmers C Miah K Murray V & Wester P O Struk turerad akutjournal för patienter med cerebrovaskular sjukdom vid medicinsk klinik Opusc Med 3 91 1978
3 – A stroke unit in a medical department Organization and the first 100 patients Acta Med Scand 205 231 1979
4 Boyle R W & Reid M What happens to the stroke victim? Geriatrics 20 949 1965
5 Bruun B & Richter R W The epidemiology of stroke in central Harlem Stroke 4 406 1973
6 Carter A B Strokes Natural history and prognosis Proc Roy Soc Med 56 483 1963
7 Cochrane A L & Holland W W Validation of screening procedures Br Med Bull 27 3 1971
8 Conant R G Perkins J A & Ainley A B Stroke morbidity mortality and rehabilitative potential J Chronic Dis 18 397 1965
9 Cooper E S Ipsen J & Brown H D Determining factors in the prognosis of stroke Geriatrics 18 3 1963
10 Dalsgaard-Nielsen T Survey of 1000 cases of apoplexia cerebri Acta Psychiat Scand 30 169 1955
11 Ericson H L The epidemiology and treatment of stroke in Lake Country Illinois Ill Med J 128 338 1965
12 Fawer R Justafre J C Berger J P & Schelling J L Intravenous glycerol in cerebral infarction a controlled 4 month trial Stroke 9 484 1978
13 Frithz G & Werner I Studies on cerebrovascular strokes II Clinical findings and short term prognosis in a stroke material Acta Med Scand 199 133 1976
14 Gilroy J & Meyer J S Vasodilator drugs in progressive cerebral infarction controlled evaluation of cerebral vasodilator drugs in the progressive stroke In Cerebral vascular disease Fifth Conf (ed C H Millikan R G Siebert and J P Whisnant) p 197 Grune and Stratton New York 1966
15 Glynn A A Vascular diseases of the nervous sys tem A series of 315 cases Br Med J 1 1216 1956
16 Kjellin K G & Soderstrom C E Diagnostic sig nificance of CSF spectrophotometry in cerebrovascu lar disease J Neurol Sci 23 359 1974
17 Lowenthal M Tobis J S & Howard I R An analysis of the rehabilitation needs and prognosis of 232 cases of cerebral vascular accident Arch Phys Med Rehabil 40 183 1959
18 Marquardsen J Natural history and prognosis of cerebrovascular disease In Cerebral arterial dis ease (ed R W Ross Russell) p 24 Churchill Living stone Edinburgh London and New York 1976
19 — The natural history of acute cerebrovascular dis ease A retrospective study of 769 patients Acta Neurol Scand (Suppl ) 38 1969
20 Mathew N T Meyer J S Rivera V M Char ney J Z & Hartman A Double blind evaluation of glycerol therapy in acute cerebral infarction Lancet 2 1227 1972
21 Melville I D & Renfrew S The prognosis of sur vival from cerebrovascular accidents J Neurol Neurosurg Psychiatry 24 346 1961
22 Oxbury J M Greenhall R C D & Grainger K M R Predicting the outcome of stroke acute stage after cerebral infarction Br Med J 3 125 1975
23 Rabkin S W Mathewson F A L & Tate R B The relation of blood pressure to stroke prognosis Ann Intern Med 89 15 1978
24 Rankin J Cerebral vascular accidents in patients over the age of 60 II Prognosis Scott Med J 2 200 1957
25 Robinson R W Demirel M & Le Beau R J Natural history of cerebral thrombosis nine to nine teen year follow-up J Chronic Dis 21 221 1968
26 Sjöström Å Hospitalized cases of strokes in a Swedish hospital region In Stroke Scandia Interna tional Symposia (ed A Engel and T Larsson) p 41 Nordiska Bokhandelns Forlag Stockholm 1967
27 Sonquist J A & Morgan J The detection of in teraction effects University of Michigan Survey Re search Center Inst for Social Research Monograph 35 Ann Arbor 1964
28 Tuthill J E Pozen T J & Kennedy F B A neurologic grading system for acute strokes Am Heart J 78 53 1969
29 Wood D H Fernbach N K & Montague M C Early predictors of stroke outcome Stroke 8 5 1977
30 Woollard M L Pearson R M Dorf G Griffith D & James I M Controlled trial of ornithine alpha ketoglutarate (OAKG) in patients with stroke Stroke 9 218 1978

Acta Med Scand 207 43–45 1980

# The Effectiveness of Clonidine as an Antihypertensive in a Two-Dose Regimen

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**ABSTRACT The therapeutic efficacy of clonidine as an antihypertensive in a b i d schedule 150–300 μg daily was evaluated The blood pressure reduction in patients with essential hypertension was satisfactory on this regimen and the steady state plasma concentrations were within the BP lowering concentration range at the end of a dosage interval**

*Key words* clonidine blood pressure

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Clonidine has proved to be effective in the treatment of mild moderate and severe hypertension A t i d dosage regimen is usually recommended (5 6) Recent studies have however shown that the plasma clearance of the drug is moderate with a terminal half life of 12 7 hours in normotensive (2) and 7 9–11 4 hours in hypertensive subjects (4) This circumstance allows a b i d administration of the drug since it has been shown that its hypotensive effect is related to its plasma concentration (0 15–2 2 ng/ml) (4)

The present investigation was undertaken to study the efficacy of the b i d regimen in patients with essential hypertension corresponding to WHO stages I–II

## PATIENTS

Eighteen patients of both sexes aged 30–61 years (mean ±S D 43±14) with essential hypertension (WHO I–II) were studied All the patients were previously untreated and recruited from a surveillance study None of the patients were menstruating at the time of investigation Further details are given in Table 1 The diagnosis of hypertension was made if three separately measured supine BPs after 15 min rest were ≥160 mmHg systolic and/or ≥95 mmHg diastolic The patients were also submitted to a physical investigation including eye fundus examination which revealed an ophthalmologic status corresponding to Keith Wagener I–II The following investigations were also carried out chest X ray ECG determination of urinary concentrations of electrolytes and catecholamines blood tests including serum electrolytes serum creatinine and creatinine clearance When indicated an intravenous pyelogram was performed

## METHODS

The patients were kept on their average diet during the investigation The initial clonidine dose was 75 μg b i d at 08 00 a m and 08 00 p m If the BP response was unsatisfactory at the next control (21 days) the dose was increased to 150 μg b i d On the day of initiation of the therapy the patient was asked to the clinic After 1 hour s rest in the recumbent position a venous sample was drawn for determination of plasma renin activity (PRA) During the corresponding 24-hour period total urine was collected for determination of creatinine clearance and urinary electrolytes At 12 months venous samples (before the morning dose) were drawn for determination of clonidine (4)

### *Blood pressure measurements*

Systolic and diastolic BPs were recorded by a mercury sphygmomanometer in the recumbent position after 15 min rest and in the standing position after 2 min Phase 5 (disappearance of Korotkoff) sound was taken as the diastolic end point Mean arterial pressure was calculated according to Rushmer (8) Readings were always made by the same person at the same time of the day 04 30–06 00 p m The patient visited the physician at the clinic 21 days 3 6 and 12 months after initiation of the therapy Mean arterial pressures <107 mmHg were regarded as a therapeutic success Clonidine was given as a standardized tablet Catapresan® The total daily dose was limited to 300 μg All patients gave their informed consent to participate in the study

### *Analysis of PRA and clonidine*

Ten ml of venous blood was drawn into prechilled tubes containing EDTA sodium and analysed according to the standardized radioimmunoassay method using the New England Nuclear kit Clonidine was analysed by gas chromatography (3) as described previously (4) in 5 ml of plasma

ments was acceptable only in patients with minor symptoms on admission

To make better prognostic assessments other significant factors have to be considered Only neurological signs were evaluated in the tested score The subjective evaluation also included in directly other clinical factors e g cardiac disease which is a very common feature in stroke patients (18 23) Several reports have stated that heart disease either unspecified (5 8) or congestive (9 25) is associated with an impaired short term prognosis after stroke It is possible that consideration of such and other factors may help to improve the quality of prognostication during and after an acute stroke

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## REFERENCES

1 Aho K Incidence profile and early prognosis of stroke Academic dissertation Helsinki 1975
2 von Arbin M Britton M de Faire U, Helmers C Miah K Murray V & Wester P O Strukturerad akutjournal för patienter med cerebrovaskulär sjukdom vid medicinsk klinik Opusc Med 3 91 1978
3 – A stroke unit in a medical department Organization and the first 100 patients Acta Med Scand 205 231 1979
4 Boyle R W & Reid M What happens to the stroke victim? Geriatrics 20 949 1965
5 Bruun B & Richter R W The epidemiology of stroke in central Harlem Stroke 4 406 1973
6 Carter A B Strokes Natural history and prognosis Proc Roy Soc Med 56 483 1963
7 Cochrane A L & Holland W W Validation of screening procedures Br Med Bull 27 3 1971
8 Conant R G Perkins J A & Ainley A B Stroke morbidity mortality and rehabilitative potential J Chronic Dis 18 397 1965
9 Cooper E S Ipsen J & Brown H D Determining factors in the prognosis of stroke Geriatrics 18 3 1963
10 Dalsgaard Nielsen T Survey of 1 000 cases of apoplexia cerebri Acta Psychiat Scand 30 169 1955
11 Ericson H L The epidemiology and treatment of stroke in Lake Country Illinois Ill Med J 128 338 1965
12 Fawer R Justafre J C Berger J P & Schelling J L Intravenous glycerol in cerebral infarction a controlled 4 month trial Stroke 9 484 1978
13 Frithz G & Werner I Studies on cerebrovascular strokes II Clinical findings and short term prognosis in a stroke material Acta Med Scand 199 133 1976
14 Gilroy J & Meyer J S Vasodilator drugs in progressive cerebral infarction controlled evaluation of cerebral vasodilator drugs in the progressive stroke In Cerebral vascular disease Fifth Conf (ed C H Millikan R G Siebert and J P Whisnant) p 197 Grune and Stratton New York 1966
15 Glynn A A Vascular diseases of the nervous system A series of 315 cases Br Med J 1 1216 1956
16 Kjellin K G & Söderström C E Diagnostic significance of CSF spectrophotometry in cerebrovascular disease J Neurol Sci 23 359 1974
17 Lowenthal M Tobis J S & Howard I R An analysis of the rehabilitation needs and prognosis of 232 cases of cerebral vascular accident Arch Phys Med Rehabil 40 183 1959
18 Marquardsen J Natural history and prognosis of cerebrovascular disease In Cerebral arterial disease (ed R W Ross Russell) p 24 Churchill Livingstone Edinburgh London and New York 1976
19 — The natural history of acute cerebrovascular disease A retrospective study of 769 patients Acta Neurol Scand (Suppl ) 38 1969
20 Mathew N T Meyer J S Rivera V M Charney J Z & Hartman A Double blind evaluation of glycerol therapy in acute cerebral infarction Lancet 2 1227 1972
21 Melville I D & Renfrew S The prognosis of survival from cerebrovascular accidents J Neurol Neurosurg Psychiatry 24 346 1961
22 Oxbury J M Greenhall R C D & Grainger K M R Predicting the outcome of stroke acute stage after cerebral infarction Br Med J 3 125 1975
23 Rabkin S W Mathewson F A L & Tate R B The relation of blood pressure to stroke prognosis Ann Intern Med 89 15 1978
24 Rankin J Cerebral vascular accidents in patients over the age of 60 II Prognosis Scott Med J 2 200 1957
25 Robinson R W Demirel M & Le Beau R J Natural history of cerebral thrombosis nine to nineteen year follow-up J Chronic Dis 21 221 1968
26 Sjöström Å Hospitalized cases of strokes in a Swedish hospital region In Stroke Scandia International Symposia (ed A Engel and T Larsson) p 41 Nordiska Bokhandelns Forlag Stockholm 1967
27 Sonquist J A & Morgan J The detection of interaction effects University of Michigan Survey Research Center Inst for Social Research Monograph 35 Ann Arbor 1964
28 Tuthill J E Pozen T J & Kennedy F B A neurologic grading system for acute strokes Am Heart J 78 53 1969
29 Wood D H Fernbach N K & Montague M C Early predictors of stroke outcome Stroke 8 5 1977
30 Woollard M L Pearson R M Dorf G Griffith D & James I M Controlled trial of ornithine alpha ketoglutarate (OAKG) in patients with stroke Stroke 9 218 1978

## ACKNOWLEDGEMENT

This study was supported by Boehringer Ingelheim AB Stockholm Sweden

## REFERENCES

1 Christensson S Frisk Holmberg M & Paalzow L Steady state concentrations of clonidine and its relation to the effects on blood pressure in normotensive and hypertensive rats J Pharm Pharmacol 31 418 1979
2 Dollery C T Davies D S Draffan G H et al Clinical pharmacology and pharmacokinetics of clonidine Clin Pharmacol Ther 19 11 1976
3 Edlund P V & Paalzow L. Quantitative gas liquid chromatographic determination of clonidine in plasma. Acta Pharmacol Toxicol 40 145 1977
4 Frisk Holmberg M Edlund P V & Paalzow L Pharmacokinetics of clonidine and its relation to the hypotensive effect in patients Br J Clin Pharmacol 6 227 1978
5 Jain A K Ryan I R Vargas R & MacMahon G F Efficacy and acceptability of different dosage schedules of clonidine Clin Pharmacol Ther 21 382 1977
6 Onesti O Schwartz A B Kim K E et al Antihypertensive effect of clonidine Circ Res (Suppl) II 53 1971
7 Raftos I Bauer G E Lewis R. G et al Clonidine in the treatment of severe hypertension Med J Aust 1 786 1973
8 Rushmer R F Cardiovascular dynamics 2nd ed Saunders Philadelphia and London 1968
9 Weber, M Case D Baer L et al Renin and aldosterone suppression in the antihypertensive action of clonidine Am J Cardiol 38 825 1976

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# A Comparative Study of Cardioselective β-Blockade and Diazepam in Patients with Acute Myocardial Infarction and Tachycardia

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ABSTRACT Eighty seven patients with an acute myocardial infarction and a pulse rate of ≥80/min on admission were randomly allotted to one group given cardioselective β-blockade, a second group given diazepam, and a third group given placebo The three groups were comparable in age, sex distribution previous history of ischemic heart disease, initial pulse rate, blood pressure pain index, enzyme values, and degree of ST elevation The acute mortality (within 10 days) did not differ between the groups The drug treatment elicited no reduction of infarct size as judged from enzyme levels degree of reduction of ST elevation, or physical exercise capacity The reasons for this negative result are discussed One possibility is that in routine clinical practice the therapeutic intervention starts too late after onset of symptoms A beneficial effect on mortality among the patients whose treatment started early after onset of symptoms supports this conclusion

*Key words* acute myocardial infarction β blockade diazepam

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Several animal experimental studies have been undertaken in recent years with the purpose of reducing the extension of an acute myocardial infarction (AMI) Interest has focused on the ischemic zone around the necrotic area Salvage of this ischemic zone by reduction of its oxygen consumption has been attempted by several experimental procedures and drugs Reduction of the infarcted area has been reported after intra aortic balloon counterpulsation (14) and administration of drugs β Receptor blocking agents such as propranolol have been studied both in experimentally induced myocardial infarction (15 19 21 22) and in human patients (20) Calcium blocking agents such as verapamil (23) infusion of glucose insulin potassium (17) hyaluronidase (16) corticosteroids (11 22) and mannitol (28) and peripherally vasodilating agents (5 10) have also been studied

Attention has focused especially on the catecholamines These elicit an increase in the myocardial oxygen consumption and are known to increase secondary to the pain and stress associated with an AMI

Melsom et al (18) showed that catecholamine excretion can be reduced in AMI by administration of diazepam Although no difference in the incidence of heart failure hypotension and cardiogenic shock was seen between the diazepam group and the control group these authors found that only the control group experienced arrhythmias such as ventricular tachycardia third degree AV block and ventricular fibrillation

The results of the influence of β receptor blockade on mortality in acute infarction are contradictory (24 25 26) Barber et al (1) found no effect of practolol on hospital mortality when comparing the treated patients with a placebo group However by selecting the patients whose heart rate exceeded 100/min they obtained a reduction of the acute mortality with practolol treatment

The present study compares the effects of diazepam and β blockade respectively with placebo in patients with an AMI and a heart rate of 80/min or more The patients of Barber et al (1) were given practolol orally and three out of four of them were admitted by a mobile coronary care unit (CCU) As mobile CCUs are not generally available the patients in the present study were given the drug intravenously immediately after admission to the CCU

*Abbreviations* AMI = acute myocardial infarction AP = angina pectoris BP = blood pressure CCU = coronary care unit ASAT = aspartate aminotransferase LD = lactic dehydrogenase a $PO_2$ = arterial oxygen tension a $PCO_2$ = arterial carbon dioxide tension

Table I *Clinical parameters in three patient groups treated with β-blockade diazepam and placebo*

| | β Blockade | | | Diazepam | | | Placebo | | |
|---|---|---|---|---|---|---|---|---|---|
| | AMI + AP | AMI | AP | AMI + AP | AMI | AP | AMI + AP | AMI | AP |
| No of admissions | 40 | 25 | 15 | 45 | 33 | 12 | 45 | 29 | 16 |
| Age (y ) | | | | | | | | | |
| $x \pm$S D | 60±8 85 | 61±7 8 | 57±10 0 | 63±9 24 | 63±9 9 | 64±7 6 | 61±8 29 | 63±7 1 | 58±9 4 |
| Range | 75–43 | 75–47 | 69–43 | 78–29 | 78–29 | 71–49 | 74–46 | 74–47 | 71–46 |
| Males ($x \pm$S D ) | 59±9 16 | 61±8 3 | 57±10 3 | 62±9 58 | 62±10 4 | 63±7 8 | 60±7 38 | 62±6 3 | 56±7 8 |
| *Females ($x \pm$S D )* | *61±7 94* | *63±5 6* | *57±11 1* | *68±5 20* | *68±5 7* | *69* | 63±10 55 | 65±10 0 | 60±11 3 |
| Male/female ratio | 32/8 | 20/5 | 12/3 | 37/7 | 26/6 | 11/1 | 33/12 | 23/6 | 10/6 |
| Hospital mortality 10 days (*n*) | 5 | 5 | 0 | 7 | 7 | 0 | 5 | 5 | 0 |
| Hospital mortality (%) | 13 | 20 | 0 | 16 | 21 | 0 | 12 | 18 | 0 |
| Mortality 11 days– 6 months (*n*) | 3 | 2 | 1 | 7 | 6 | 1 | 2 | 2 | 0 |
| Total mortality (*n*) | 8 | 7 | 1 | 14 | 13 | 1 | 7 | 7 | 0 |
| Hours from onset to drug | | | | | | | | | |
| $x \pm$S D | 23±32 8 | 28±39 6 | 16±12 0 | 20±19 4 | 20±19 5 | 21±20 2 | 20±29 6 | 16±18 4 | 27±42 |
| Median | 11 | 10 | 15 5 | 13 | 13 | 18 | 9 5 | 18 | 8 5 |
| History of previous infarct (yes/no) | | 6/19 | | | 7/25 | | | 7/22 | |

## PATIENTS AND METHODS

Patients referred to the CCU of Malmo General Hospital with a suspicion of an AMI were included in the study providing a heart rate of 80 beats/min or more was recorded 5 min after application of the precordial ECG electrodes and the start of an i v infusion of 5 5% glucose The heart rate was calculated from the QRS complexes visible on the oscilloscope screen Patients were excluded if at the time of counting the heart rate they had 2nd or 3rd degree AV block pulmonary edema or cardiogenic shock (blood pressure (BP) less than 80 mmHg during half an hour and in addition pale clammy skin or cerebral confusion)

The diagnosis of AMI was based on the following criteria 1) central thoracic pain combined with two elevated enzyme values aspartate aminotransferase (S-ASAT) and/or lactic dehydrogenase (S LD) *myocardial isoenzymes* 1 and/or 2 2) central thoracic pain and WHO electrocardiographic infarction criteria and 3) ECG changes and two elevated enzyme values

Serum enzymes S-ASAT and S ALAT were measured daily for the first three days S LD including isoenzymes was measured on the third day after admission If the clinical condition so required more determinations of these enzyme values were made

Arterial oxygen (a $PO_2$) and carbon dioxide (a $PCO_2$) tension were measured on the second day after admission as described earlier (8) and again if the clinical condition so required Oral temperature was measured twice daily 0 5°C was added to the oral value BP was recorded indirectly with the cuff method Heart rate was measured by counting QRS complexes for 30 sec from the ECG strips A 12 lead ECG (direct writing ink jet Mingograph 62 Siemens Elema Solna Stockholm Sweden) was recorded 5 min after the start of the i v infusion and application of electrodes 20 min after drug injection and in the morning of each of the first 7 days The electrode site was indicated with a small ink spot to ensure an unchanged position at *the various ECG recordings*

BP was measured and pain index recorded at the same time as the 12 lead ECG Pain index was estimated by one of the nurses or doctors who were unaware of the drug given according to the following grading 0=no pain

Table II *Enzyme blood gas and body temperature values in the three AMI patient groups*

| | β Blockade | | | Diazepam | | |
|---|---|---|---|---|---|---|
| | $x$ | $n$ | S D | $x$ | $n$ | S D |
| S ASAT initial–max value (μkat/l) | 1 28–4 79 | 25–25 | 1 61–4 89 | 1 31–3 76 | 32–30 | 1 73–2 71 |
| S-ALAT initial–max value (μkat/l) | 0 63–2 20 | 25–25 | 0 46–3 89 | 0 60–1 06 | 32–30 | 0 46–0 69 |
| S-LD max value (μkat/l) | 27 5 | 22 | 22 4 | 28 6 | 28 | 19 10 |
| a $PO_2$ 2nd day–min value (kPa) | 9 4–8 4 | 19–6 | 3 23–2 29 | 10 0–10 2 | 19–4 | 2 30–4 61 |
| a $PCO_2$ *2nd day–min* value (kPa) | 5 3–4 9 | 19–6 | 1 26–0 53 | 5 1–4 7 | 19–4 | 0 74–1 02 |
| Temperature initial–max value (°C) | 37 2–38 1 | 16–25 | 0 62–0 60 | 37 1–38 0 | 21–29 | 0 61–0 62 |

1=slight retrosternal oppression 2=easy constant pain 3=moderate pain 4=severe pain 5=very intense retrosternal pain

All patients were monitored immediately after admission to the CCU for 1–3 days depending on the clinical condition The number and amount of arrhythmias observed on the oscilloscope screen were recorded by specially trained nurses

The patients were allocated to one of three groups 1) Practolol (Eraldina® ICI) 20 mg as a slow i v injection for 5 min followed by 50 mg of atenolol (Tenormin® ICI) orally twice daily for 7 days 2) Diazepam (Stesolid® Dumex) 5 mg as a slow i v injection for 5 min followed by 15 mg three times a day orally for 2 days and 5 mg three times a day for a further 5 days 3) Saline as a slow i v injection for 5 min followed by placebo tablets three times a day for 7 days Oral administration was started just after the second ECG recording on the day of admission Besides this regimen the patients were treated with antiarrhythmics diuretics and digitalis according to ward routine

The patient was allocated to one of the three treatment groups depending on the date of birth Those born on the 1st 4th 7th 10th 31th day of the month were given β-blockade those born on the 2nd 5th 8th 11th 29th diazepam and those born on the 3rd 6th 9th 12th 30th placebo

The heart was X rayed before the patients left the hospital Heart volume in the standing position was determined according to Jonsell (9) Relative heart volume (ml/m² BSA) was estimated according to Lysholm et al (13)

Just before and three months after the patients left the hospital an exercise test was performed on those aged 65 or less with the patient sitting on an electrodynamically braked bicycle ergometer They started with a work load of 25 J for 6 min The load was then increased to 50 J for a further 6 min and to 75 J if the patient could tolerate this The exercise was stopped if 1) angina pectoris (AP) appeared or 2) the pulse rate rose to more than 130/min or 3) ventricular tachycardia (3 ventricular premature beats or more) appeared or 4) the patient became tired and therefore could not continue The maximum intensity work load was determined as described earlier (8) as the heaviest load at which the patient worked for 6 min with an increment proportional to the completed part of the six minute period at the load higher than this

The significance of differences between groups and of paired differences within groups was evaluated by Student's *t* test

## RESULTS

The total series comprises 130 patients of whom 87 were diagnosed as having AMI and 43 AP Table I gives the age and sex distribution and the interval between onset of symptoms and initiation of drug treatment

The three AMI series were comparable in age and sex distribution (although the mean age of the β blockade patients was slightly but not significantly lower) and also in the number of previous infarctions which was 6/25 in the β blockade group 8/33 in the diazepam group and 7/29 in the placebo group

Table II lists the serum enzyme and blood gas values and the body temperature No significant differences were found for either initial or maximum values

The mean pulse ratio/min at rest in the β blockade group was 92 diazepam group 93 and placebo group 99 (Table III) These differences were not significant ($p>0$ 05) Corresponding values 20 min after injection were 79 91 and 100 beats/min 7 days after the start of treatment 69 85 and 78 10 days after the start of treatment 75 84 and 82 The β blockade resulted in a significant decrease in pulse rate during the treatment period but the value in the β blockade group 10 days after the start of treatment is not significantly lower than that in the diazepam and placebo groups ($p>0$ 05)

The mean systolic BP before administration of drug was 153 mmHg in the β blockade group 150 in the diazepam group and 144 in the placebo group (Table III) These differences were not significant ($p>0$ 05) Corresponding values 20 min after drug administration were 142 145 and 143 mmHg This drop was not significant in any group ($p>0$ 05) The systolic BPs in all three groups decreased until the third day when they levelled off and remained virtually unchanged for the rest of the period The drop in systolic BP was of the same magnitude in all three groups

The mean diastolic BP in the patients on β blockade was 93 on diazepam 96 and on placebo 91 mmHg (Table III) These differences are not significant ($p>0$ 05) Corresponding figures 20 min after drug administration were 88 93 and 89 The diastolic BP in the β blockade group levelled off on the third day and in the other two groups on the

| Placebo | | |
|---|---|---|
| | *n* | S D |
| 58–4 96 | 29–28 | 4 19–5 60 |
| 4–3 18 | 29–28 | 7 18–8 58 |
| 4 | 27 | 16 80 |
| 7–6 0 | 22–6 | 1 85–1 37 |
| 7–5 4 | 22–6 | 0 62–0 49 |
| 7 1–38 0 | 23–28 | 0 74–0 71 |

Table III *Pulse rate systolic and diastolic BP and pain index in patients with AMI and on different drug regimens*

| | β Blockade | | | Diazepam | | | Placebo | | |
|---|---|---|---|---|---|---|---|---|---|
| | x | n | S D | x | n | S D | x | n | S D |
| Pulse rate/min | | | | | | | | | |
| Before drug | 92 | 25 | 13 6 | 93 | 33 | 16 3 | 99 | 29 | 20 4 |
| 20 min after drug | 79 | 25 | 9 9 | 91 | 33 | 15 3 | 100 | 28 | 20 7 |
| 7 days of treatment | 69 | 20 | 20 0 | 85 | 22 | 14 2 | 78 | 23 | 12 9 |
| 10 days after start of treatment | 75 | 16 | 12 8 | 84 | 20 | 15 0 | 82 | 19 | 12 2 |
| Systolic BP (mmHg) | | | | | | | | | |
| Before drug | 153 | 25 | 33 6 | 150 | 30 | 31 9 | 144 | 27 | 35 0 |
| 20 min after drug | 142 | 22 | 28 6 | 145 | 29 | 28 2 | 143 | 26 | 31 8 |
| 7 days of treatment | 117 | 15 | 21 3 | 117 | 17 | 10 5 | 122 | 21 | 23 0 |
| Diastolic BP (mmHg) | | | | | | | | | |
| Before drug | 93 | 25 | 18 3 | 96 | 30 | 18 1 | 91 | 27 | 20 5 |
| 20 min after drug | 88 | 22 | 15 9 | 93 | 29 | 17 6 | 89 | 26 | 18 1 |
| 7 days of treatment | 71 | 19 | 8 9 | 75 | 17 | 9 1 | 76 | 21 | 14 5 |
| Pain index | | | | | | | | | |
| Before drug | 1 2 | 18 | 1 6 | 1 6 | 24 | 1 6 | 1 5 | 22 | 1 8 |
| 20 min after drug | 1 2 | 17 | 1 6 | 1 0 | 23 | 1 3 | 1 3 | 20 | 1 6 |
| 7 days of treatment | 0 2 | 15 | 0 6 | 0 1 | 16 | 0 3 | 0 | 16 | 0 |

fourth thereafter the values remained virtually unchanged Also the drop in diastolic BP was of the same magnitude in all three groups

The pain index mean value before drug administration in the β blockade group was 1 2 in the diazepam group 1 6 and in the placebo group 1 5 (Table III) These differences are not significant ($p>0\ 05$) *Corresponding values 20 min after drug* were 1 2 1 0 and 1 3 The decrease in the diazepam group was significantly lower ($0\ 01<p<0\ 05$) than in the β blockade group No differences in pain index were observed in the groups during the rest of *the study*

The mean S T segment elevation in leads $V_1$–$V_5$ and $V_7$ in the β blockade group was 0 95 mV diazepam group 0 74 and placebo group 0 76 before drug administration These differences were not significant ($p>0\ 05$) Corresponding values 20 min *after injection were 0 73 0 57 and 0 71 The S T* elevation remained on this level during the rest of the study Similar values were found when S T elevations in all 12 leads were measured When those of leads $V_1$–$V_7$ were selected in which the S T elevation was ≥0 2 mV the mean value before injection in the β blockade group was 0 49 diazepam group 0 43 and placebo group 0 43 mV These differences are not significant ($p>0\ 05$) Corresponding values 20 min after injection were 0 40 0 38 and 0 41 mV and on the 10th day 0 37 0 29 and 0 31 mV respectively The drop was not significant when the values obtained before and 20 min after drug administration were *compared When the* comparison was made with the second-day values the drop was significant in the β blockade and placebo groups ($0\ 01<p<0\ 05$) but not in the diazepam group ($p>0\ 05$)

There were no major differences in the mean number of arrhythmic episodes *in the three groups*

Table IV *Arrhythmias in patients with AMI and on different drug regimens*

SVPB = supraventricular premature beat VPB = ventricular premature beat VT = ventricular tachycardia VF = ventricular fibrillation

| | β-Blockade | Diazepam | Placebo |
|---|---|---|---|
| Monitoring time (h) | 125 | 95 | 87 |
| No of episodes with SVPB/hour | | | |
| 1–10 | 20 | 17 | 37 |
| 11–30 | 0 | 23 | 56 |
| >30 | 0 | 0 | 2 |
| No of episodes with VPB/hour | | | |
| 1–10 | 27 | 32 | 24 |
| 11–30 | 25 | 2 | 14 |
| >30 | 0 | 0 | 0 |
| No of episodes with VT | 4 | 0 | 1 |
| No of episodes with VF | 1 | 1 | 1 |

as far as ventricular premature beats are concerned (Table IV) The number of supraventricular premature beats was smaller in the $\beta$ blockade and diazepam groups than in the placebo group Ventricular fibrillation was observed once in each group

Side effects were especially looked for Pulmonary edema occurred once in each of the diazepam and placebo groups but not in the $\beta$ blockade group Corresponding figures for left ventricular failure defined as bilateral rales over at least 1 dm of the lung bases were 5 3 and 2 Third degree AV block was seen in 2 patients in the diazepam group but in neither of the other two groups

The sedative effect of diazepam was especially pronounced in the older patients and could give rise to nursing problems All patients were given the same physical therapy with breathing instructions and leg movements as a thrombosis prophylaxis Three patients in the $\beta$ blockade group had physical findings of pneumonia none in the diazepam group and one in the placebo group Corresponding figures for a radiological finding of pneumonia were 3 2 and 1 There are four drop outs in the $\beta$ blockade group (cardiac decompensation 3 BP drop 1) four in the diazepam group (too heavy sedation) and none in the placebo group

An X ray of the heart just before the patient left the hospital did not reveal any larger heart volume in the $\beta$ blockade group than in the other two groups The total volume was 887 ml and the relative volume 489 ml/m² BSA Corresponding values for the diazepam group were 959/530 and for the placebo group 1090/571 These differences were not significant

The amount of additional drugs needed was calculated for the three groups There was no significant difference between the three groups in the amount of analgesics given nor did the number of patients who needed analgesics differ There was no significant difference between the groups in the amount of antiarrhythmic drugs and diuretics administered

Hospital mortality did not differ significantly between the three groups being 20 21 and 17% in the $\beta$ blockade diazepam and placebo group respectively Neither was there any significant difference in mortality during the first six months although this was higher in the diazepam group 39% than in the $\beta$ blockade 28% and placebo groups 24%

The exercise test revealed no difference between the groups in total work load at discharge from hospital After three months the total work load was slightly higher in the diazepam group 82 64 J than in the placebo group 63 07 J ($0\ 05<p<0\ 01$) The value in the $\beta$ blockade group 75 01 J did not differ significantly from the others There were no significant changes between the groups in pulse or BP response

## DISCUSSION

The pathophysiological mechanisms behind the arrhythmias in the acute phase of myocardial infarction are not known in detail Several mechanisms are probably involved in the different stages after infarction The increased sympathetic tone and the raised plasma concentrations of catecholamines are among the factors held responsible for the occurrence of serious complications such as malignant arrhythmias left ventricular failure and cardiogenic shock (7) The present study was done in patients with a tachycardia in order to get a maximum beneficial result of the therapeutic intervention Because even a mild increase in heart rate led to a striking increase in infarct size in closed chest dogs (4) the limit was set at a pulse rate of 80/min

The present study shows no significant differences between the patients with AMI concerning mortality enzyme values or ST elevations when treated with $\beta$ blockade diazepam and placebo

The three series were comparable in age sex distribution previous history of ischemic heart disease initial pulse rate BP pain index and enzyme values Furthermore, Malmo with a population of 243 591 inhabitants in 1976 is served by only one hospital This means that all patients with an AMI are brought there which excludes selection of the material

A double blind technique is not feasible for a study which compares the effect of $\beta$ blockade and diazepam because of the drop in pulse rate and sedative effect respectively For practical reasons the date of birth was used for grouping the patients This resulted in a complete randomization of relevant parameters

It is advantageous to administer selective $\beta$ adrenergic receptor blockers to AMI patients because in some acutely ill patients it can be difficult on admission to get a reliable history for example concerning obstructive lung disease To reduce the extension of the infarction it is mandatory to administer the drug as early as possible Practolol

produces only minor hemodynamic effects (6) therefore this drug was selected for i v administration Practolol because of its long term side effects (2) is not available in tablet form in Sweden therefore atenolol also a selective $\beta$ blocking agent was afterwards administered orally

Several end points were used to evaluate the effect of the drugs There was no difference between the groups when a hard end point such as mortality was applied The hospital mortality during the first ten days after infarction for the groups with $\beta$ blockade, diazepam and placebo was 20 21 and 17%, respectively Although drug was administered only during seven days after the infarction the patients were followed up for six months The additional mortality during this period was two six and two making a total mortality for the six months of 28 39 and 24% respectively

It is possible that a hard end point such as mortality is too crude a measure More delicate but also clinically less clear cut end points such as the degree of ST elevation enzyme values and results of an exercise test might give an indication that the therapy salvaged some of the myocardium but not enough to reduce mortality However no statistically significant differences between the three groups were found for S ASAT S LD or body temperature Theoretically this finding could be explained by assuming that the infarcts of the placebo group were smaller than those of the two other groups But no statistically significant differences were observed in the initial values recorded just after admission rather there was a tendency to a higher S ASAT and a lower a $PO_2$ value in the placebo group A previous study showed that a low a PO carries a poor prognosis (12) Also no more pronounced drop of the ST elevation was observed in the $\beta$ blockade group than in the other groups This was so both when the ST elevation in all the 12 leads and in the six precordial leads was calculated also when only the precordial leads with an ST elevation $\geq 0$ 2 mV were included

A bicycle ergometer exercise test was performed just before the patient left the hospital and three months after One third of the patients in each of the three groups participated If part of the myocardium had been salvaged one could expect a better physical exercise capacity in the treated groups compared with the placebo group But no such difference was found

A drug induced decrease in the myocardial work would result in a reduction of chest pain in patients with an AMI This has indeed been claimed (27) but could not be confirmed in the present study (Table III) Although the dosage of practolol differed in the two studies the pulse reduction was of the same magnitude In addition the patients experience of pain was evaluated in the present study from the total amount of analgesics given no statistically significant difference was found between the three groups

Although the ischemic twilight zone around the region of central necrosis after an experimentally induced infarct in animals has been claimed to continue to enlarge for up to 18 hours after occlusion (3) it is apparent that the interval between the onset of the infarction process and the initiation of drug administration is of major importance when trying to reduce the extent of the infarct The median duration of drug administration in the present patients was 10 13 and 18 hours in the $\beta$ blockade diazepam and placebo groups respectively Although the median duration was longer in the placebo group the mortality was not higher A difficulty that arises is to decide when the infarction starts Although the onset of symptoms is usually connected with the onset of infarction this is open to discussion Furthermore in some patients with multiple attacks of chest pain it may be difficult to decide which of these attacks announces the infarction

The present study suggests that the time elapsing between the onset of infarction and the initiation of drug administration in routine clinical practice is often too long for a significant reduction of infarct size To study the effect of early drug administration from each group patients were selected to whom the drug was given within four hours after onset of symptoms The acute ($\leq$10 days) or late (10 days–6 months) mortality in the $\beta$ blockade group was 0/5 and 0/5 whereas 2/6 acute and 2/4 late deaths occurred in the diazepam group Corresponding figures in the placebo group were 1/5 and 0/4 The acute mortality among those to whom drug administration started 4–8 hours after onset of symptoms was 1/6 1/5 and 2/4 in the three groups respectively Corresponding figures for the late mortality were 0/5 0/4 and 1/2 Although the figures are small, they indicate a beneficial effect of $\beta$ blockade in AMI patients with tachycardia when treatment starts within eight hours after onset of symptoms

In conclusion there is no indication from this material to administer β receptor blocking agents routinely to patients with an AMI when arriving at the CCU Although the differences were not significant there was a tendency to a lower age lower pulse rate lower heart volume and shorter interval between onset of symptoms and drug administration in the β blockade group than in the placebo group yet the mortality in the β blockade group was 20% against 17% in the placebo group However when the interval between onset of symptoms and initiation of drug administration is short eight hours or less a certain beneficial effect cannot be excluded from the outcome of the present study

## REFERENCES


1 Barber J M McBoyle C Chaturvedi N C Singh N & Walsh M J Practolol in acute myocardial infarction Acta Med Scand 587 213 1975
2 Conolly M E Kersting F & Dollery C T The clinical pharmacology of beta adrenoceptorblocking drugs Prog Cardiovasc Dis 19/3 203 1976
3 Cox J L McLaughlin V W Flowers N C & Moran L G The ischemic zone surrounding acute myocardial infarction its morphology as detected by dehydrogenase staining Am Heart J 76 650 1968
4 Editorial Beta blockade and size of acute myocardial infarction Lancet 2 813 1974
5 Epstein S E Hypotension nitroglycerin and acute myocardial infarction Circulation 47 217 1973
6 Gibson D G & Coltart D J Haemodynamic effects of practolol Br Heart J 34 95 1972
7 Jewitt D E Nercer C J Reid D Valori C Thomas M & Shillingford J P Free noradrenaline and adrenaline excretion in relation to the development of cardiac arrhythmias and heart failure in patients with acute myocardial infarction Lancet 1 635 1969
8 Johansson B W Complete heart block A clinical hemodynamic and pharmacological study in patients with and without an artificial pacemaker Acta Med Scand (Suppl) 180 1966
9 Jonsell S A method for the determination of the heart size by teleroentgenography (a heart volume index) Acta Radiol (Stockh) 20 325 1939
10 von Leitner E R Kötter V & Schroder R Beeinflussung der Infartkgrosse durch Senkung der Herzarbeit bei akutem Myokardinfarkt Z Kardiol (Suppl) 2 61 1975
11 Libby P Maroko P R Bloor C M Sobel B E & Braunwald E Reduction of experimental myocardial infraction size by corticosteroid administration J Clin Invest 52/I 599 1973
12 Ljungstrom B Johansson B W & Sievers J Arterial $pO_2$ $pCO_2$ pH and standard bicarbonate in patients with an acute myocardial infarction Cardiologia 51 138 1967
13 Lysholm E Nylin G & Quarnå K The relation between the heart volume and stroke volume under physiological and pathological conditions Acta Radiol (Stockh) 15 237 1934
14 Maroko P R Bernstein E F Libby P De Lana G A Covell J W Ross J Jr & Braunwald E Effects of intra aortic balloon counterpulsation on the severity of myocardial ischemia injury following acute coronary occlusion counterpulsation and myocardial injury Circulation 45 1150 1972
15 Maroko P R & Braunwald E Modification of myocardial infarction size after coronary occlusion Ann Intern Med 79 720 1973
16 Maroko P R Libby P Bloor C M Sobel B E & Braunwald E Reduction of hyaluronidase of myocardial necrosis following coronary artery occlusion Circulation 46 430 1972
17 Maroko P R Libby P Sobel B E Bloor C M Sybers H D Shell W E Covell J W & Braunwald E Effect of glucose insulin potassium infusion on myocardial infarction following experimental coronary artery occlusion Circulation 45 1160 1972
18 Melsom M Andreassen P Melsom H Hansen T Grendahl H & Hillestad L K Diazepam in acute myocardial infarction Clinical effects and effects on catecholamines free fatty acids and cortisol Br Heart J 38 804 1976
19 Miura M Ganz W Thomas R Singh B N Sokol I & Shell W E Reduction of infarct size by propranolol in closed-chest anesthetized dogs Circulation (Suppl) II 159 1976
20 Pitt B Weiss J L Schulze R A Taylor D R Kennedy H L & Coralls D Reduction of myocardial infarct extension in man by propranolol Abstract Circulation (Suppl) II 11 1976
21 Reimer K A Rasmussen M M & Jennings B B Reduction by propranolol of myocardial necrosis following temporary coronary artery occlusion in dogs Circulation (Suppl) II 11 1976
22 Shatney C H Mc Carter D J & Lillehei R C Effects of allopurinol propranolol and methylprednisolone on infarct size in experimental myocardial infarction Am J Cardiol 37 572 1976
23 Smith H J Singh B N Heather D Nisbet H D & Norris R M Effects of verapamil on infarct size following experimental coronary occlusion Cardiovasc Res 9 569 1975
24 Snow P J D Effect of propranolol in myocardial infarction Lancet 2 551 1965
25 — Treatment of acute myocardial infarction with propranolol Am J Cardiol 18 458 1966
26 Sowton E Beta adrenergic blockade in cardiac infraction Prog Cardiovasc Dis 10 561 1968
27 Waagstein F & Hjalmarson Å C Effect of cardioselective beta blockade on heart function and chest pain in acute myocardial infarction Acta Med Scand (Suppl) 587 193 1975
28 Willerson J T Powell W Gutney T L Stark J J Sanders C A & Leaf A Improvement in myocardial function and coronary blood flow in ischemic myocardium after mannitol J Clin Invest 51/II 2989 1972

# The Relationship between Marginal Bone Loss and Serum Zinc Levels

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**ABSTRACT** Serum was analyzed for zinc in 51 patients of varying age and with varying degrees of alveolar bone loss as recorded on roentgenograms There was a reversed correlation between marginal alveolar bone loss and serum zinc levels The observations are discussed in relation to the physiological functions of zinc

*Key words* periodontal disease zinc
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In a detailed medical survey of a few patients with advanced periodontal disease some extremely low serum zinc levels were recorded The present study was performed in order to find out if any reversed correlation actually existed between the degree of periodontal disease and the serum zinc levels in a larger material As other laboratory tests on blood samples from patients with periodontal disease usually only reveal levels within the normal range we presumed that our observations might be of some interest

## SUBJECTS AND METHODS

The study comprises 21 males and 30 females Their age and sex distribution is presented in Table I The subjects were recruited by four different dentists among patients attending general dental and periodontal care in four different private dental clinics and among patients from the University Clinic of Periodontology According to the routine interview the participants were presumably healthy

### *Evaluation of marginal bone loss*

Intraoral roentgenograms were used for determining an index of marginal bone loss (MBL) (7 14) For each tooth excluding the wisdom teeth the mesial and the distal alveolar bone loss was evaluated on roentgenograms The distance from the alveolar crest to the apex of the tooth was determined in per cent of the distance from the cementoenamel junction to the apex A modification of a ruler described by Schei et al (20) was used for the measurements (Fig 1) Eleven equispaced radii are drawn on a translucent plastic ruler The radii are given numbers 0–10 A number of lines indicating the inclination of the ruler at the measurement are drawn perpendicular to the middle radius The ruler was applied over the roentgenogram with the radius 0 covering the cementoenamel junction approximally projected and moved until radius 10 covered the apex MBL value 1 was noted when the alveolar crest was visible between radii 0 and 1 MBL value 2 when the alveolar crest was visible between radii 1 and 2 and so on In cases where the alveolar crest could be seen under a radius line the higher score was chosen The point of the alveolar crest referred to is the junction between the lamina dura dentis and interdental bone The mean value of all recordings from each patient is called the MBL index

### *Serum analysis*

Venous blood 10 ml was drawn from each participant and allowed to clot Blood was centrifuged at about 3000 r p m for 5 min to obtain serum The serum sample was inspected and if the slightest haemolysis was observed the specimen was discarded All glass tubes were acid cleaned and thoroughly rinsed in deionized water The concentration of zinc in serum was analyzed mostly within 24 hours in an atomic absoption spectrophotometer type Perkin Elmer 306 Separate tests were made to ensure that the glass tubes and stoppers used did not contribute any measurable amount of zinc to the specimens

## RESULTS

The mean serum zinc level of the 51 participants was 0 73 ppm (S D 0 099) In Fig 2 each serum zinc level is plotted against the recorded MBL index The mean MBL was 2 92 (S D 1 57) The statisti

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Table I *Data on the 51 subjects studied (group means)*

| Age group | Males | Females | Serum zinc (ppm) | MBL index | r | No of subj with serum Zn ≥0 80 ppm |
|---|---|---|---|---|---|---|
| 20–30 | 3 | 7 | 0 731 | 1 97 | −0 33 | 4 |
| 30–40 | 6 | 9 | 0 747 | 2 38 | −0 17 | 6 |
| 40–50 | 5 | 7 | 0 731 | 3.51 | −0 55 | 5 |
| 50– | 7 | 7 | 0 719 | 3 71 | −0 43 | 1 |
| Total | 21 | 30 | 0 732 | 2 92 | −0 37 | 16 |

cal analysis indicates that the correlation between serum zinc concentration and MBL is −0 37 and is significant on the 1% level Correlations were also calculated separately for each age group (Table I) Sixteen participants with a mean serum zinc level of 0 80 ppm or higher had a mean MBL of 1 96 Serum zinc levels below 0 80 ppm in 35 subjects corresponded to a mean MBL of 3 37 The normal range of serum zinc with the present method is 0 80–1 25 ppm

## DISCUSSION

The participants in the present study were selected by four of the authors during a period of about six months with the primary aim of making up the age groups with patients with various degrees of periodontal disease Periodontal patients were untreated or in various stages of treatment Periodontal surgery had not been performed within 3 weeks prior to their participation The study population is markedly selected and is not meant to be representative of the general population

Periodontitis is an extremely common chronic disease characterized by a gradual loss of the tooth supporting tissues as a result of a more or less pronounced inflammatory reaction to the bacterial plaque deposited on the tooth surfaces close to the gingiva In the general population periodontitis results in a significant increase of MBL with advancing age (14) a fact which is also reflected in the present material (Table I)

The human body contains 1 4–2 3 g Zn the largest amounts being localized in muscle bone and skin (1) Zinc is also present in the plasma erythrocytes leucocytes and platelets (23) For clinical purposes it is usually analyzed in serum

The normal serum zinc levels given by different authors vary greatly Disintegration of platelets during clotting and haemolysis results in higher serum zinc recordings There are also geographical differences in the normal zinc levels (11) which might be explained by dietary factors The influence of age and sex on normal plasma and serum zinc levels as

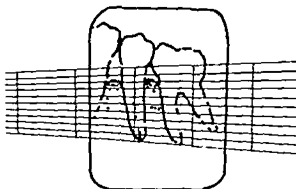


*Fig 1* Ruler used for measurement of MBL on roentgenograms Tooth 35 has distally an MBL value of 2

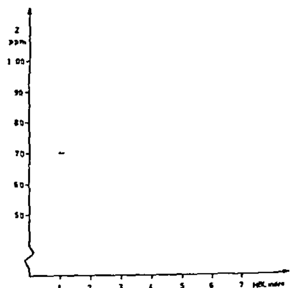



*Fig 2* Serum zinc levels plotted against the recorded MBL index for each patient

reported by several investigators was reviewed by Chooi et al (2) They concluded that up to the age of 50 the mean plasma zinc of normal individuals remains relatively constant while above 50 it decreases significantly with age

Our subjects did not show any significant decrease in serum zinc levels with age (Table I) The reversed correlation between serum zinc and MBL index can therefore not be related to the influence of age Various degrees of zinc deficiency seem to occur even in presumably well nourished societies (23) as a result of an increased excretion due to decreased absorption and utilization

A large number of diseases and conditions are associated with a decreased serum zinc level (19) They include infectious diseases (16) surgical trauma (17 22) and burns (13) To the best of our knowledge periodontal disease has not previously been discussed in relation to the physiological functions of zinc

Zinc occurs in all types of cells and body fluids and is known to be a vital part of about 20 metalloenzymes (18) It participates in almost all physiological processes Its functions are frequently related to the presence of other trace elements (8)

The concentration of zinc in serum seems to have several regulating functions The aggregability of platelets is decreased (4) and the release of histamine is inhibited (9 10) by increasing the content of zinc in serum Also some functions of macrophages (25) and granulocytes (4) are inhibited by zinc administration Chvapil et al (5) demonstrated in vitro that increased zinc levels in the presence of magnesium reversibly inhibited various functions of dog peripheral granulocytes and that this function is closely associated with zinc uptake by the cells The functions influenced were $O_2$ consumption phagocytosis and bactericidal activity The depression of serum zinc values which occurs as a result of a redistribution within the body a few hours after infection coincides with enhancement of some neutrophil functions (24) It has also been suggested (3) that zinc ions have a membrane stabilizing function with increasing concentrations of zinc there is a reduced release of lysosome content and a decreased mobility of the inflammatory cells

The chronic inflammatory periodontal lesion associated with progressive destruction of periodontal tissues is an extremely complex reaction Most of the evidence indicates that the presence of plaque initiates immunopathologic and other destructive inflammatory mechanisms (15 21) One main feature is the decreased content of collagen in affected tissue A part of the collagen destruction is probably due to the release of enzymes from the membrane coated lysosomes in the inflammatory cells (12) There are also indications that the loss of connective tissue substance may be a consequence of depressed collagen production (21) Fernandez Madrid et al (6) showed that in zinc-deficient rats the deposition of collagen in healing wounds is defective

In view of the regulatory functions of zinc in the inflammatory process and in collagen metabolism there are reasons to believe that the low serum zinc level in patients with periodontal disease may be a factor of clinical importance that deserves further attention

## REFERENCES


1 Beisel W R Pekarek R S & Wannemacher R W Jr Homeostatic mechanisms affecting plasma zinc levels in acute stress In Trace elements in human health and disease vol I (ed A S Prasad and D Oberleas) p 87 Academic Press New York 1976
2 Chooi M K Todd J K & Boyd N D Influence of age and sex on plasma levels in normal and diabetic individuals Nutr Metab 20 135 1976
3 Chvapil M New aspects in the biological role of zinc A stabilizer of macromolecules and biological membranes *Life Sci 13 1041 1973*
4 — Effect of zinc on biomembranes and cells Med Clin North Am 60 4 1976
5 Chvapil M Stankova L Zukoski C IV & Zukoski C III Inhibition of some functions of polymorphonuclear leukocytes by in vitro zinc J Lab Clin Med 89 135 1977
6 Fernandez Madrid F Prasad A S & Oberleas D Effect of zinc deficiency on collagen metabolism J Lab Clin Med 78 853 1971
7 Henrikson C O & Lavstedt S Precision and accuracy in intraoral roentgenological determination of proximal marginal bone loss Acta Odontol Scand (Suppl) 33 26 1975
8 Hill C H Mineral interrelationships In Trace elements in human health and disease vol II (ed A S Prasad and D Oberleas) p 281 Academic Press New York 1976
9 Kazimierczak W & Maslinski C Histamine release from mast cells by compound 48/80 The membrane action of zinc Agent Action 4 320 1974
10 — The effect of zinc ions on selective and nonselective histamine release in vitro Agent Action 4 1 1974
11 Kubota J Lazar V A & Loose F Copper zinc cadmium and lead in human blood from 19 locations

in the United States Arch Environ Health 16 788 1966
12 Lange D Bang J & Cimasoni G Cytochemical demonstration of lysosomal enzymes in human crevicular fluid J Dent Res 50 756 1971
13 Larson D L Maxwell R Abston S & Dobrkovsky M Zinc deficiency in burned children Plast Reconstr Surg 46 13 1970
14 Lavstedt S & Eklund G Some factors of significance for proximal marginal bone loss studied on an epidemiological material Acta Odontol Scand (Suppl) 33 50 1975
15 Niesengard R J The role of immunology in periodontal disease J Periodontol 48 505 1977
16 Oon B B Khong K Y Greaves M W & Plummer V M Trophic skin ulceration of leprosy Skin and serum zinc concentrations Br Med J 2 5918 1974
17 Pories W J Mansour E G Plecha F R Flynn A & Strain W H Metabolic factors affecting zinc metabolism in the surgical patient In Trace elements in human health and disease vol I (ed A S Prasad and D Oberleas) p 115 Academic Press New York 1976
18 Riordan J F & Vallee B L Structure and function of zinc metalloenzymes In Trace elements in human health and disease vol I (ed A S Prasad and D Oberleas) p 227 Academic Press New York 1976
19 Sandstead H H Vo-Khactu K P & Solomons N Conditioned zinc deficiencies In Trace elements in human health and disease vol I (ed A S Prasad and D Oberleas) p 33 Academic Press New York 1976
20 Schei O Waerhaug J Lövdal A & Arno A Alveolar bone loss as related to oral hygiene and age J Periodontol 30 7 1959
21 Schluger S Yuodelis R A & Page R C Periodontal disease chapter 8 Lea & Febiger Philadelphia 1977
22 Sefton G Clark R G & Owen G Changes in serum zinc after operation Br J Surg 61 329 1974
23 Underwood E J Trace elements in human and animal nutrition pp 202 228 Academic Press New York 1977
24 Wannemacher R Pekarek R S Klainer A Bartelloni P Dupont H Hornick R & Beisel W Detection of a leucocytic endogenous mediator like mediator of serum amino acid and zinc depression during various infectious illnesses Infect Immun 11 873 1975
25 Zukoski C F Chvapil M Carlson E Hattler B & Ludwig J Functional immobilization of peritoneal macrophages by zinc J Reticuloendothel Soc 16 6a 1974

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# Bone Mineral Content in Women with Vertebral Fractures

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**ABSTRACT** Postmenopausal women classified as osteoporotics on the basis of clinical and microradiographic findings were found to have a low bone mineral content as compared with age matched controls The bone mineral content in 12 patients on a dietary supplement of calcium and vitamin D did not decrease during a two-year follow up period

*Key words* bone calcium

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The vertebral crush fracture syndrome is a clinical entity which often runs a characteristic course After an episode of back pain the patient becomes asymptomatic for a long period

This phenomenon raises the question of whether patients with this history have persistently increased bone loss or whether they cease to lose bone in periods of remission To elucidate the pattern of bone loss in relation to vertebral fractures repeated bone mineral measurements were made in various parts of the skeleton in a number of post menopausal women with spinal fractures and the clinical diagnosis of osteoporosis

## SUBJECTS AND METHODS

The study comprised 19 postmenopausal women with one or more vertebral fractures which had occurred spontaneously or after minor trauma The patients had been classified as osteoporotics on the basis of clinical and microradiographic findings

The bone mineral content was determined in four parts of the skeleton as described below Twelve patients were treated with a dietary supplement of calcium (Calcium Sandoz® 0 5 g one tablet twice daily) and vitamin D (500 IU daily) for a period of two years The change in bone mineral content during this treatment was determined by repeated measurements at intervals of 3-6 months

For comparison the bone mineral content was determined once in age matched controls without clinical signs of vertebral fractures The control group comprised 10 female staff members and 9 women drawn at random from the Stockholm population The age was 63±6 years (mean±S D ) in the control group and 63±5 years in the fracture group

The bone mineral content was determined by X ray spectrophotometry (4 5) In this method the bone under examination is positioned by TV fluoroscopy and automatically scanned by a beam at two energy levels from an X ray tube The attenuation of the beam in the bone is recorded as a profile giving the mineral content in mg/mm The forearm (radius + ulna 1 and 8 cm proximal to the radiocarpal joint) and the femur (neck and shaft) were chosen These measuring sites have been described in detail elsewhere (3) The statistical calculations were made according to Student s *t* test

## RESULTS

The osteoporotic women with vertebral fractures were found to have a lower bone mineral content than the controls although the difference was significant only in the femur (Table I) Repeated measurements revealed no decrease in the bone mineral content in the osteoporotics during a two-year follow up period (Table II)

## DISCUSSION

The great overlap in bone mineral content between subjects with and without fractures made it difficult to evaluate the grade of osteoporosis in individual cases The variation in mineral content could be reduced in the control group by correcting age but even then there was no clear difference in mineral content at any measuring sites between individuals with and without fractures

However the group with vertebral fractures had a significantly lower bone mineral content in the femur than the controls The low mineral content may explain why patients with vertebral fractures are prone to repeated fractures of the hip and the wrist (6) It is also consistent with the finding that spinal fractures are related to a low total body calcium (2) Apparently weight bearing parts of the

Table I *Bone mineral content (mg/mm) in different parts of the skeleton in 19 osteoporotic women with vertebral fractures and in 19 controls*

| | Controls | | Osteoporotics | | Difference | | |
|---|---|---|---|---|---|---|---|
| | Mean | S D | Mean | S D | % | *t* | *p* |
| Radius + ulna | | | | | | | |
| Distal | 86 | 18 | 79 | 18 | −8 | 1 20 | >0 05 |
| *Shaft* | 142 | 25 | 134 | 24 | −6 | 1 01 | >0 05 |
| Femur | | | | | | | |
| Neck | 252 | 34 | 221 | 47 | −12 | 2 33 | <0 05 |
| Shaft | 427 | 70 | 374 | 67 | −12 | 2 38 | <0 05 |

*skeleton are not protected against osteoporosis as indicated by the low mineral content in the femur*

The prospective part of the study did not reveal any loss of bone mineral in the osteoporotics during the observation period of two years This confirms the results of Buring et al (1) and Shapiro et al (7) who found a constant mineral content in the forearms of women with spinal fractures during treatment with calcium and vitamin D On these grounds it is reasonable to conclude that osteoporotics may cease to lose bone mineral for long periods without any other therapy than dietary supplements

Table II *Annual change (%) in bone mineral content in 12 osteoporotic women with vertebral fractures calculated from repeated measurements during a two year follow up period*

| | Mean | S D | *p* |
|---|---|---|---|
| Radius + ulna | | | |
| Distal | +2 4 | 5 7 | >0 05 |
| Shaft | −0 6 | 4 6 | >0 05 |
| Femur | | | |
| Neck | +0 9 | 6 2 | >0 05 |
| Shaft | −1 0 | 3 4 | >0 05 |
| Mean of four sites | +0 4 | 2 7 | >0 05 |

## REFERENCES


1 Buring K Hulth A G Nilsson B E Westlin N E & Wiklund P E Treatment of osteoporosis with vitamin D Acta Med Scand 195 471 1974

2 Cohn S H Ellis K J Wallach S Zanzi I Atkins H L & Aloia J F Absolute and relative deficit in total skeletal calcium and radial bone mineral in osteoporosis J Nucl Med 15 428 1974

3 Dalén N & Jacobson B Bone mineral assay Choice of measuring sites Invest Radiol 9 174 1974

4 Gustafsson L Jacobson B & Kusoffsky L X ray spectrophotometry for bone mineral determinations Med Biol Eng 12 113 1974

5 Jacobson B X ray spectrophotometry in vivo Am J Roentgenol Radium Ther Nucl Med 91 202 1964

6 Saville P D The syndrome of spinal osteoporosis Clin Endocrinol Metabol 2 177 1973

7 Shapiro J R Moore W T Jorgensen H Reid J *Epps H & Whedon D Osteoporosis Evaluation of* diagnosis and therapy Arch Intern Med 135 563 1975

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# Familial Occurrence of the Haemolytic Uraemic Syndrome

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ABSTRACT A family is described in which the haemolytic uraemic syndrome (HUS) occurred in two generations Both juvenile and adult onset of this syndrome were observed in this family Those affected were all women three developed HUS in the postpartum period one towards the end of pregnancy and one as a five-year old child Because five cases were observed over a period of 16 years, exposure to the same infectious agent is highly unlikely Although the transmission of a 'dormant virus cannot be excluded, the occurrence of HUS in two generations of one and the same family seems compatible with the hypothesis that susceptibility to the disease is transmitted as an autosomal dominant characteristic This observation suggests a genetic influence on the development of HUS possibly in conjunction with other factors, such as infectious agents pregnancy and/or delivery

*Key words* familial haemolytic uraemic syndrome
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Haemolytic uraemic syndrome (HUS) is a combination of microangiopathic haemolysis thrombocytopenia and acute renal failure caused by local intravascular coagulation in the kidney probably due to exposure of subendothelial structures after the endothelium has been damaged by an agent of unknown aetiology (12) The syndrome is more common in children but also occurs in adults especially in women during the postpartum period (2 3 7 14 18 19) A familial occurrence of the juvenile and adult forms has been described previously (1 6 11)

To our knowledge this is the first report of a family with HUS which was manifested in the post partum period in more than one generation

## CASE REPORTS

Clinical and laboratory data are outlined in Table I and the family pedigree is shown in Fig 1

*Case 1*

This 18-year-old woman (III 23) delivered her second child in 1969 One month before delivery slight proteinuria was found in the absence of hypertension and oedema One month after delivery she complained of headaches fever and a swelling of the right cheek supposedly due to a dental infection Treatment with penicillin gave initial improvement but one week later the headaches increased and vomiting occurred Pallor periorbital oedema and hypertension (160/105 mmHg) were noted on admission Heart liver and spleen were not enlarged ESR was 65 mm in the first hour The Hb concentration was 7 5 g/dl WBC 6000/mm³ platelet count 80000/mm³ The blood smear showed anisocytosis poikilocytosis and fragmentocytosis SLDH was elevated (1150 IU/l) serum creatinine 13 8 mg/dl and bilirubin 0 6 mg/dl Proteinuria was present (1 4 g/24 h) The urinary sediment showed 10–20 red cells per high-power field The bleeding time was prolonged (10–13 min Ivy) Prothrombin time and fibrinogen concentration were normal Antiglobulin tests and ANF were negative

She developed oliguria and haemodialysis was initiated The renal insufficiency appeared to be irreversible One year later bilateral nephrectomy was performed and she received a cadaveric kidney which is still functioning satisfactorily 9 years after transplantation

Microscopic examination of the kidneys revealed some intimal fibrosis in the arteries The glomeruli showed tuft collapse and some endothelial swelling The capillary basement membranes were wrinkled not split The tubules showed some atrophy and a few hyaline casts some were dilated These findings were though to be compatible with acute ischaemia as can be seen in HUS

*Case 2*

In 1959 this 5 year-old girl (III 22) was admitted to hospital for pain in the abdomen vomiting and epistaxis She had never been ill before Her BP was 190/140 mmHg and

*Fig 1* Pedigree of the family

body temperature slightly elevated (38°C) Her body was covered with petechiae and an occasional ecchymosis The liver was moderately enlarged the spleen could not be palpated The Hb concentration was 5 5 g/dl reticulocyte count 500 000/mm³ The blood smear showed schistocytes and helmet and burr cells The bone marrow preparation revealed hyperactive erythropoiesis and adequate platelet production BUN was 126 mg/dl There was marked proteinuria The urinary sediment contained 15–20 red cells per high-power field and numerous granular casts The bleeding time was prolonged (7½ min) The prothrombin time was normal

She received blood transfusions and antibiotics and a protein restricted diet was prescribed Nevertheless BUN rose to 163 mg/dl and she became oliguric Peritoneal dialysis was initiated A biopsy from the right kidney showed lesions that were interpreted as being irreversible Therefore as was usual at that time haemodialysis was not continued Subsequently she became comatose and died 4 weeks later

Microscopic examination of the kidneys disclosed concentric intimal fibrosis with local fibrinoid changes in many of the arteries The glomeruli showed tuft collapse with focal necrosis and ghost glomeruli with a decrease in the number of nuclei the glomerular basement membrane was split locally The tubules and interstitium showed minor changes Microangiopathic renal disease was diagnosed (Figs 2 and 3)

*Case 3*

This woman (II 5) was 38 years old when she delivered her 5th child in 1966 Because signs of mastitis developed one week after delivery she was treated with tetracycline and stilboestrol Five weeks later she started to complain of fatigue swelling of the face hands and feet and the occurrence of ecchymoses Pallor jaundice and oedema of the periorbita and ankles were evident on admission BP was 200/105 mmHg The heart was enlarged liver and spleen were not ESR was 42 mm in the first hour Hb concentration was 4 9 g/dl WBC 4 000/mm³ The blood smear showed marked anisocytosis and polychromasia The platelet count was 56 000/mm³ reticulocyte count 110 000 mm³ There was marked proteinuria (12‰ Esbach) The urinary sediment contained 10 red cells per high-power field and a few hyalinized casts Serum creatinine was 3 45 mg/dl on the first day and 18 mg/dl several days later Unconjugated bilirubin was elevated and haptoglobulin was low The antiglobulin tests (Coombs ) both direct and indirect were negative ANF was negative Clotting time and prothrombin time were normal but the bleeding time was prolonged (15 min Ivy) Fibrinogen concentration was decreased (95 mg/dl) The

Table 1 *Clinical and laboratory findings on admission*

| | Case no | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Age (y ) | 18 | 5 | 38 | 40 |
| Weeks postpartum | 4 | | 5 | 6 |
| BP (mmHg) | 160/105 | 190/140 | 200/105 | 220/130 |
| Blood urea (mg/100 ml) | 280 | 260 | 75 | 280 |
| Hb (g/100 ml) | 7 5 | 5 5 | 6 3 | 7 1 |
| Reticulocytes (‰ RBC) | 110 | 270 | 107 | 90 |
| RBC fragmentation | + | + | + | ? |
| Platelets/mm³ | 80 | 15 | 56 | 80 |
| Haemorrhagic signs | − | + | + | + |



*Fig 2* To the left of the glomerulus the vascular pole with endothelial proliferation The capillary tuft shows swollen endothelial cells and some mesangial increase In the upper right corner an arteriole with intimal proliferation (silvermethenamine HE ×195)



*Fig 4* On the left the vascular pole and the macula densa Thrombus in the vascular pole (HE ×195)

bone marrow smear showed active erythropoiesis and sufficient megakaryopoiesis

Peritoneal dialysis was started She developed motoric unrest and seizures The cerebrospinal fluid was normal the EEG showed diffuse abnormalities During the 3rd peritoneal dialysis extensor cramps and coma occurred and she died

Microscopic examination of the kidneys disclosed arteries with concentric intimal fibrosis glomeruli with focal local hyperaemia and capillary thrombi There was some increase in the intracapillary cells A focal glomerulus was necrotic Oxalate protein and erythrocyte and granulocyte casts were seen in the tubules In the interstitium there was some leukocyte infiltration and fibrosis The diagnosis was acute pyelonephritis and microangiopathic renal disease (Fig 4)



*Fig 3* Glomerulus with proximal tubule The capillary walls show extensive mesangium interposition (splitting) and there is mesangial hyperplasia (silvermethenamine HE ×195)

*Case 4*

This 40-year-old woman (II 4) the mother of cases 1 and 2 became ill 6 weeks after delivering her 7th child in 1961 All previous pregnancies and deliveries had been uneventful Her symptoms were fatigue headaches and vomiting Three days later a haemorrhagic diathesis developed and she became soporous Oedema in the orbital region and around the ankles was noted on admission Large ecchymoses were present on the arms and legs BP was 270/130 mmHg the left ventricle was enlarged ESR was elevated (106 mm in the first hour) there was anaemia (Hb 5 5 g/dl) with mild reticulocytosis (100000/mm³) and thrombocytopenia (80000 mm³) WBC was 4500/mm³ There was proteinuria the urinary sediment contained numerous leukocytes and an occasional cellular cast BUN was 130 mg/dl

She developed oliguria and subsequently anuria The haemorrhagic diathesis was considered to be an impediment to haemodialysis at that time and she died 10 days after the first onset of symptoms

Autopsy was performed in another centre (University Hospital Nijmegen Holland) The renal autopsy specimens were examined and compared with the specimens from her younger sister (II 5) The histological findings were similar and compatible with microangiopathic renal disease

*Case 5*

In 1953 in the last trimester of her 4th pregnancy after 3 uncomplicated pregnancies this 37 year-old woman (II 3) developed serious generalized oedema and hypertension in the course of one week The urine contained a large amount of protein She became oliguric and died on admission to hospital Further clinical information and autopsy results are not available

## DISCUSSION

The main reason for studying a family such as the one described here is the increasing interest in ge

netics in clinical medicine A systematic investigation of the family histories of patients with renal failure has indicated that the proportion of genetically determined renal diseases is higher than anticipated amounting at present to about 20% of the entire patient population in our dialysis and transplantation centre Whether the observed familial incidence of HUS is due to genetic factors remains however uncertain

We assume that all five women described suffered from HUS In three patients (II 4 II 5 III 23) the clinical picture and the circumstances under which the disease occurred are highly similar All three became ill 4–6 weeks after an uncomplicated delivery As regards case II 3 the diagnosis of HUS is uncertain but it might be inferred from the rapidly progressive renal failure that developed after 8 months of an uneventful pregnancy The diagnosis of HUS was well established in patient III 22 who has been described previously (16)

All three postpartum patients suffered from a microangiopathic haemolytic anaemia and a moderately severe thrombocytopenia

Rapidly progressive renal insufficiency and hypertension malignant in two cases were present and contributed to the death of two patients The results of the histological examination of the kidneys were compatible with microangiopathic thrombosis fibrosis of the intima fibrinoid material in the arterioles microthrombi and swelling of intracapillary cells in the glomeruli were seen

The clinical course laboratory data and histological findings make the diagnosis of HUS highly probable in four out of the five cases

Other diagnoses that were considered but could be ruled out were acute tubular necrosis and acute glomerulonephritis Eclampsia occurring more than 48 hours after delivery is less severe than that occurring during delivery and has not been described in connection with acute renal failure Malignant hypertension could be excluded because severe signs of renal insufficiency preceded the hypertension in three patients

The pathogenesis of HUS is not known but local intravascular coagulation with deposits of fibrin like material in arterioles and glomerular capillaries seems to play an important role in the course of the renal failure (9 12) Because it is so markedly localized the intravascular coagulation is probably triggered by the exposure of subendothelial structures (collagen) to the blood stream

The aetiological factor that causes the endothelial lesion could be an infectious agent a toxin or the immune response to such an agent The specificity of the infectious agent that can cause HUS appears to be low In children in particular various viral and bacterial infections have been suggested (9 10 11 15 17) Epidemiological data and the fact that respiratory and intestinal infections as well as vaccinations have been found to precede HUS support the theory that infectious agents play an aetiologic role The role of infectious agents is less clear in the adult onset type Two of our patients (II 5 and III 23) presumably had had a bacterial infection shortly before the HUS appeared Several authors suggest drugs like oxytocin and ergometrine (19) oral contraceptives (5) and certain antibiotics such as tetracycline One of our patients (II 5) was treated with tetracycline and oestrogens before HUS became manifest

The similarity between HUS and the Shwartzman phenomenon in the animal model is considered striking (8) but endotoxin has not been demonstrated in humans The study by Koster et al (13) is an exception to this rule Interesting in this respect is the fact that pregnancy can facilitate the occurrence of the Shwartzman phenomenon in animals Whether inhibition of the fibrinolytic mechanism or blockade of the reticuloendothelial system plays a role in such a situation is not clear

Apart from environmental factors and the role played by pregnancy and/or delivery a genetic predisposition might contribute to the outbreak of the disease Kaplan et al (11) however point out that exogenous influences might cause a familial occurrence

The time lapse between the cases described here and the occurrence in two generations support the hypothesis that a genetic predisposition plays an important role in the pathogenesis of the disease in this family

We wonder how unique this family with HUS in childhood and in the postpartum period really is As pointed out before the growing interest in medical genetics may lead to discovery of similar families and thus the possibility of follow up studies

A discussion of the controversy regarding early treatment of HUS (5) is beyond the scope of this communication Our main purpose was to call attention to a factor which may contribute toward the development of HUS i e genetic predisposition

Whether pregnancy should be avoided in this family to prevent the risk of maternal HUS or the transmission of possibly lethal genetic material is a matter for debate

## REFERENCES


1 Blatter W Wegmann W Herold H et al Familiares hamolytisch uramisches Syndrom Schweiz Med Wochenschr 105 1773 1975
2 Churg J Koffler D Paronetto F et al Haemolytic uremic syndrome as a cause of post partum renal failure Am J Obstet Gynecol 108 253 1970
3 Clarkson A R Lawrence J R Meadows R et al The haemolytic uraemic syndrome in adults Q J Med 154 227 1970
4 Editorial Haemolytic ureamic syndrome of young women Lancet 1 943 1976
5 Editorial Haemolytic ureamic syndrome in childhood Lancet 1 26 1978
6 Farr M J Roberts S Morley A R et al The haemolytic uraemic syndrome—a family study Q J Med 174 161 1975
7 Finkelstein F O Kashaearian M & Hayslett J P Clinical spectrum of postpartum renal failure Am J Med 57 649 1974
8 Gaynor F Bouvier C & Spaet T H Vascular lesions possible pathogenetic basis of the generalized Shwartzman reaction Science 170 986 1971
9 Gervais M Richardson J B Chin J et al Immunofluorescent and histological findings in the haemolytic uraemic syndrome Pediatrics 47 352 1971
10 Gianantonio C Vitacco M Mendilaharzu F et al Haemolytic uraemic syndrome J Pediatr 64 478 1964
11 Kaplan B S Chesney R W & Drummond K N Haemolytic uraemic syndrome in families N Engl J Med 292 1090 1975
12 Kaplan B S & Drummond K S The haemolytic syndrome is a syndrome N Engl J Med 298 964 1978
13 Koster F Levin J Walker L et al Hemolytic uremic syndrome after shigellosis Relation to endotoxemia and circulating immune complexes N Engl J Med 298 927 1978
14 Luke R G Siegel R R & Talbert W Heparin treatment for post partum renal failure with microangiopathic anemia (letter) Lancet 1 804 1971
15 Mettler N E Isolation of a microtatobiote from patients with haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura and from mites in the United States N Engl J Med 281 1029 1969
16 Monnens L & Retera R J M Varianten van het haemolytisch-uraemisch syndroom bij kinderen Maandschr Kindergeneesk 33 205 1965
17 Ray C G Tucker V L Harris D J et al Enteroviruses associated with the hemolytic uremic syndrome Pediatrics 46 378 1970
18 Robson J S Martin A M Ruckley V A et al Irreversible post partum renal failure A new syndrome Q J Med 37 423 1968
19 Wagoner R D Holley K E & Johnson W J Accelerated nephrosclerosis and post partum acute renal failure in normotensive patients Ann Intern Med 69 237 1968

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# Peripheral Blood Flow in Chronic Ergotism

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**ABSTRACT** Muscle blood flow (MBF) was determined in 11 patients on chronic overdoses of ergotamine tartrate and in 12 controls using the local $^{133}$xenon clearance method The difference in MBFs between the groups was insignificant 59 7±21 4 and 61 6±10 8 ml/100 g/min, respectively Six patients taking ergotamine in doses exceeding 0 25 mg/kg/week showed a significant ($p<0\ 01$) reduction of MBF (45.2±10 7 ml/100 g/min) This reduction could be measured before the manifestation of symptoms or signs of circulatory insufficiency

*Key words* ergotamine headache migraine peripheral circulation xenon clearance

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Chronic ergotism due to consumption of rye bread contaminated with Claviceps purpurea was endemic in the Middle Ages Most modern cases are iatrogenic caused by prescribed ergot derivatives in the treatment of migraine Peripheral arteriospasm predominantly in the lower limbs is a well recognized complication of ergotamine treatment seen even after therapeutic doses (1 2 5 9 13) Little is known however about the peripheral circulation in subclinical ergotism (4)

Skeletal muscle blood flow (MBF) can be evaluated quantitatively by measuring the disappearance of $^{133}$xenon from the muscle (7) Being a lipophilic gas $^{133}$Xe diffuses so freely between muscle tissue and blood that a diffusion equilibrium is practically maintained regardless of the rate of blood flow For such a tracer the blood flow is the only limiting factor in removal from the muscle

The aim of this study is to evaluate using the $^{133}$Xe clearance method the possible impairment of peripheral circulation in patients taking excessive amounts of ergotamine tartrate

## PATIENTS AND METHODS

The study was carried out in 11 patients suffering from severe migraine The mean age was 37 5 years (range 23–61) They had been taking at least 10 mg of ergotamine tartrate weekly mostly as suppositories for at least one year The patients had no cardiovascular symptoms such as intermittent claudication Three of them complained of mild distal numbness The physical examination was unremarkable in all cases Peripheral pulses were palpable and there was no other evidence of circulatory insufficiency The control group consisted of 12 healthy volunteers aged 23–55 years (mean 36 9)

All subjects were studied in the supine position and they had not smoked for at least 2 hours Studies were performed at room temperature (22–25 C) and the subjects had rested for 30 min before the study started A detailed description of the methods is given elsewhere (8) In brief $^{133}$Xe 50–100 μCi dissolved in isotonic saline (not more than 0 1 ml) was injected into the anterior tibial muscle via a narrow gauge needle as atraumatically as possible Special care was taken not to inject any air bubbles into the muscle The reactive hyperemia was induced by complete ischemia using a cuff pressure (>220 mmHg) above the knee with simultaneous dorsiplantar flexions of the ankle for 3 min The disappearance of $^{133}$Xe was monitored at 10-second intervals by a scintillation detector with a 1 75×2 inch NaI crystal connected to a multichannel analyzer (Nokia Lp 4840) The MBF was calculated by the equation of Lassen et al (7) $MBF = k_x \times \lambda \times 100$ ml/100 g/min where $k_x$ is the slope of the best fit line giving the fractional loss of tracer per unit of time and λ is the partition coefficient for xenon between muscle and blood a value of 0 7 was used (3)

The study was performed in both limbs in all cases and the MBF values displayed a random side to-side difference The coefficient of variation was 5 5–22 5% (mean 12 0) An exceptionally low MBF value considered to be an artifact due to deposition of the injected solution in fatty tissue was measured in one leg of one patient Therefore only the higher value was used in the calculations

## RESULTS

The maximal hyperemia occurred in both groups during 30–90 sec after the release of the cuff pressure and the MBF values were calculated from this

This study was presented in part at the Joint Meeting of the Italian Headache and Scandinavian Migraine Societies Florence Italy June 2–3 1976

Table I *Clinical details and MBF of the patients*
Patients 1–4 7 8 whose ergotamine doses exceeded 0 25 mg/kg/week had significantly reduced MBF values compared to the controls (45 2±10 7 vs 61 6±10 8 ml/100 g/min $p<0\ 01$ Student's $t$ test)

| Pat no | Sex | Age (y) | Weight (kg) | MBF (ml/100 g/min) | Ergotamine tartrate Dosage/week mg | mg/kg | Duration of use (y) | Symptoms[a] |
|---|---|---|---|---|---|---|---|---|
| 1 | ♀ | 23 | 55 | 42 6 | 50–60 | 0 91–1 09 | 3 | |
| 2 | ♀ | 40 | 48 | 41 3 | 15–30 | 0 31–0 62 | 5 | Numbness |
| 3 | ♀ | 56 | 58 | 30 1 | 15–16 | 0 26–0 28 | 1 | Numbness |
| 4 | ♀ | 35 | 51 | 42 0 | 14–15 | 0 27–0 29 | 8 | Numbness |
| 5 | ♀ | 30 | 57 | 76 4 | 14 | 0 25[a] | 2 | |
| 6 | ♀ | 61 | 60 | 78 3 | 12–13 | 0 20–0 22 | 7 | |
| 7 | ♀ | 30 | 50 | 57 5 | 14 | 0 28 | 2 | |
| 8 | ♂ | 29 | 76 | 58 2 | 25–28 | 0 33–0 37 | 5 | |
| 9 | ♂ | 27 | 74 | 67 4 | 10–14 | 0 14–0 19[b] | 1 | |
| 10 | ♂ | 46 | 70 | 105 3 | 10–11 | 0 14–0 16 | 2 | |
| 11 | ♂ | 46 | 72 | 57 8 | 10–11 | 0 14–0 15 | 1 | |

[a] Only symptoms suggestive of peripheral circulatory impairment are listed
[b] Medication had been discontinued one week before the study

period There was no significant difference in MBF values between ergotamine overdose patients (59 7±21 4) and controls (61 6±10 8) Six patients who were taking ergotamine tartrate in higher doses than 0 25 mg/kg/week showed a significant ($p<0\ 01$) reduction of MBF (45 2±10 7 ml/100 g/min) Individual results of the patients are given in Table I

## DISCUSSION

The [133]xenon method is established in the clinical evaluation of peripheral blood flow (7 10) and the measured MBF values correlate well with the true blood flow measured by direct venous outflow measurements (11) Furthermore the MBF values correlate closely with clinical and angiographic findings (10) When the [133]Xe method is used in clinical studies it is essential that the studied muscle is hyperemic because the resting MBF values in normal and abnormal limbs do not differ significantly (7 10)

Ergotamine and its derivatives have a direct vasoconstrictor action and an indirect vasodilator action through sympathetic ganglion blockade (12) Clinically recognizable vasoconstriction is usually not encountered unless large doses are used but patients differ remarkably in ergot tolerance In severe cases moreover extensive intimal lesions and thrombi of the smaller arteries are features of ergotism and contribute to the ischemia induced by the vasoconstriction (13) The histology of an unobliterated artery is different the arterial lesions are segmental and they involve essentially the media and adventitial layer while the intima is well preserved (6) The observed impairment of peripheral blood flow could be a manifestation of these changes narrowing the arterial lumen

The present results agree with those of a recent study by Dige Petersen et al (4) They showed using strain gauge plethysmography low normal or reduced foot systolic blood pressures in asymptomatic ergotamine overdose patients In a group of patients who were able to discontinue their ergotamine intake the distal blood pressure rose significantly being normal from the ninth day This observation is consistent with the clinical impression that the ergotamine induced arteriospasm is reversible if thrombosis has not occurred Angiographic follow up studies (1 2 5 6 9) have also shown regression of initial vasospastic changes even the subsidence of prominent collaterals (5 6) after discontinuation of ergotamine intake

In conclusion the ergotamine overdose patients tolerate the toxic effects of the drug remarkably well even prolonged use of massive doses (e g patient 1) but chronic use seems to lead to a dose dependent reduction of peripheral blood flow These patients are usually symptom free or have only mild and non specific symptoms Because the physical examination particularly the pulse palpa

tion is not sufficiently accurate an additional objective test is needed to evaluate the possible impairment of peripheral circulation The local $^{133}Xe$ clearance method is a useful non invasive test for this purpose

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## REFERENCES

1 Ahlgren I Haeger K Nylander G & Wehlin L Imminent gangrene of the leg after ergot poisoning Angiology 19 354 1968
2 Bertho E Ratte J Jean J D & Gagnon J C Iatrogenic ergotism Report of a case Angiology 20 455 1969
3 Conn H L Jr Equilibrium distribution of radioxenon in tissue Xenon hemoglobin association curve J Appl Physiol 16 1065 1961
4 Dige Petersen H Lassen N A Noer I Tönnesen K H & Olesen J Subclinical ergotism Lancet 2 65 1977
5 Enge I & Sivertssen E Ergotism due to therapeutic doses of ergotamine tartrate Am Heart J 70 665 1965
6 Fievez M Philippart F & Hustin J Ergotism Anatomoclinical study of a case Angiology 26 491 1975
7 Lassen N A Lindbjerg J & Munck O Measurement of blood flow through skeletal muscle by intramuscular injection of Xenon 133 Lancet 1 686 1964
8 Leinonen H Salminen S & Peltokallio P Capillary permeability and maximal blood flow in skeletal muscle in athletes and non athletes measured by local clearances of $^{133}Xe$ and $^{131}I^-$ Scand J Clin Lab Invest 38 223 1978
9 Merhoff G C & Porter J M Ergot intoxication Historical review and description of unusual clinical manifestations Ann Surg 180 773 1974
10 Miller T A Lindenauer S M & Pozderac R V $^{133}$Xenon clearance in the diagnosis of arterial occlusive disease J Surg Res 16 412 1974
11 Tonnesen K H & Sejrsen P Washout of $^{133}$xenon after intramuscular injection and direct measurement of blood flow in skeletal muscle Scand J Clin Lab Invest 25 71 1970
12 Ulrich J & Sigaard Andersen J Vascular effects of dihydrogenated ergot alkaloids Angiology 22 622 1971
13 Yater W M & Cahill J A Bilateral gangrene of feet due to ergotamine tartrate used for pruritus of jaundice JAMA 106 1625 1936



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# $\alpha_1$-Antitrypsin and other Acute Phase Reactants in Liver Disease

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**ABSTRACT The plasma acute phase reactant pattern was studied in 124 patients with liver disease and 16 healthy individuals undergoing liver biopsy $\alpha_1$ Antitrypsin levels were found to correlate positively with the extent of hepatocellular damage, inflammatory activity and total biopsy score Haptoglobin levels correlate negatively with these parameters and particularly with characteristics conducive to portal hypertension Orosomucoid and fibrinogen were unaffected by extent of disease and activity These changes result in a typical acute phase reactant pattern, seen most frequently in viral hepatitis and chronic active hepatitis and less frequently in alcoholic liver disease When present, it has a high specificity and predictive value for detection of liver disease**

*Key words* $\alpha_1$ antitrypsin acute phase reactants liver disease biopsy

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Increased levels of $\alpha_1$ antitrypsin ($\alpha_1$ AT) have been reported in individuals of normal Pi phenotype in various diseases of the liver (11 12) Kindmark and Laurell (5) have demonstrated a particular pattern (high $\alpha_1$ AT normal orosomucoid low haptoglobin) of acute phase reactant proteins in cases of viral hepatitis B differing from the typical acute phase reactant pattern found in other inflammatory conditions

In the present study plasma levels of $\alpha_1$ AT and other acute phase reactants are correlated to the extent of change in biopsy characteristics using an objective scale for the evaluation of concomitant liver biopsies Clear diagnostic groups are compared statistically The pattern of acute phase reactant proteins regarded as typical for liver disease is defined and its predictive significance is evaluated

## STUDY POPULATION AND METHODS

The original material comprises 202 consecutive liver biopsies taken when clinically indicated from patients admitted to Malmö General Hospital Forty six patients had alcoholic liver disease (ALD) (16 with Laennec s cirrhosis (LC) and 28 alcoholic hepatitis (AH)) Thirteen patients had primary biliary cirrhosis (PBC) 18 chronic active hepatitis (CAH) 5 chronic persistent hepatitis (CPH) 2 acute viral hepatitis (VH) 2 cryptogenic cirrhosis (CC) in advanced stages and 4 venous congestion (VC) Thirty four additional patients had primary liver disease of unclear or complex type Sixty two patients who had either malignancy or systemic disease influencing the plasma protein pattern or whose biopsy specimens were too small for reliable interpretation were excluded

Fasting EDTA plasma samples were obtained on the morning after Menghini needle liver biopsy Control biopsies were obtained with written patient consent and with the approval of the Ethical Committee of the Medical Faculty University of Lund from 16 subjectively healthy patients with normal liver enzymes during elective cholecystectomy The final study population thus consisted of 140 patients 16 of whom were healthy controls In addition plasma protein patterns of 17 patients with VH (no biopsy) were included for comparison

All biopsies were evaluated using only the internal code from the Department of Pathology for identification Biopsies were judged using Scheuer s Liver Biopsy Interpretation (10) as reference Each biopsy was given 0–3 points for each of the characteristics bridging necrosis cirrhosis fibrosis round cell portal infiltration piecemeal necrosis spotty lobular necrosis bile duct replication cholestasis Kupffer cell activity and subjective inflammatory activity (2) The latter was roughly judged as follows 0 = no inflammation 1 = one-cell necrosis with round cell infiltration 2 = infiltration of round cells leucocytes and macrophages and 3 = diffuse inflammation of all cell types (13) Additional consideration was given extreme anisocytosis anisokaryosis and degree of hy

*Abbreviations* $\alpha_1$ AT = $\alpha_1$ antitrypsin ALD = alcoholic liver disease LC = Laennec s cirrhosis AH = alcoholic hepatitis PBC = primary biliary cirrhosis CAH = chronic active hepatitis CPH = chronic persistent hepatitis VH = viral hepatitis CC = cryptogenic cirrhosis VC = venous congestion

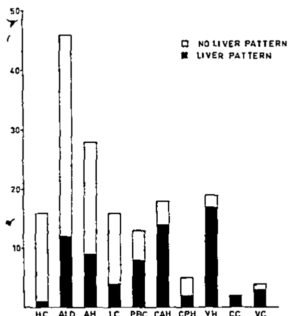



*Fig 2* Presence of the acute phase reactant pattern in different liver disease groups *HC* = healthy controls

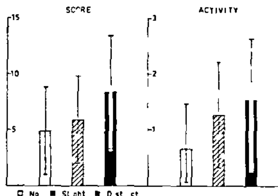



*Fig 3* Increasing liver biopsy score and activity found in patients with no slight and distinct patterns of acute phase reactants Patterns are slight if $\alpha_1$ AT minus orosomucoid ≈ 5–14% and $\alpha_1$ AT minus haptoglobin ≥20% distinct if $\alpha_1$ AT minus orosomucoid ≥15% and $\alpha_1$ AT minus haptoglobin ≥30%

gen Haptoglobin concentrations decrease sharply and significantly ($p \leq 0\ 01$) but with great variation between individuals with increase in these biopsy characteristics

Regression lines were similarly calculated for the relationships between $\alpha_1$ AT orosomucoid and haptoglobin and other biopsy characteristics The results shown in Table II demonstrate significant increases in $\alpha_1$ AT in necrotic and inflammatory conditions and with increasing Kupffer cell activity Haptoglobin decreases with increasing fibrosis cirrhosis portal inflammation and spotty lobular necrosis Orosomucoid covaries only with increasing level of steatosis

A comparison of $\alpha_1$ AT concentrations in different liver disease groups and controls is given in Table III Highly significant increases in plasma $\alpha_1$ AT were found in cases of AH and VH significant increases in all forms of ALD and CAH slight increases in PBC and no significant change in VC or CC Using the criteria above for the pattern of acute phase reactants typical for liver disease frequencies of the pattern in the various diagnostic groups are shown in Fig 2 The frequency of the pattern occurs in the following order VH>CAH>PBC>AH>alcoholic cirrhosis In Fig 3 the acute phase pattern has been subdivided into groups with smaller and greater slopes to demonstrate the tendency toward increased biopsy score and activity in patients with slight and distinct acute phase patterns

Ceruloplasmin is generally accepted as an indicator of oestrogen effect in the plasma protein pattern (7) To evaluate possible oestrogen effect contributing to the acute phase pattern in liver disease average ceruloplasmin values were calculated in patients with and without the acute phase pattern Average values were 0 41 g/l (±0 13 $n$=48) and 0 44 g/l (±0 13 $n$=76) respectively No difference in ceruloplasmin levels between those with and without the acute phase pattern could be seen among patients with cholestasis

The clinical usefulness of the acute phase reactant pattern was investigated using 250 consecutive plasma protein analyses and reviewing the patients charts Sensitivity specificity and the predictive value of the pattern were calculated (Table IV) Of the 250 patients 69 had a history of current acute or chronic liver disease and/or pathological liver enzymes Of these 69 patients 20 satisfied the criteria for the acute phase reactant pattern

In addition 8 patients without history of liver disease had the pattern In this group 2 patients with metastases of mammary cancer were receiving treatment with the oestrogen receptor blocking

Table IV *Sensitivity specificity and predictive value of the acute phase reactant pattern typical of liver disease*

TP = true positive FP = false positive FN = false negative TN = true negative

| | Pats with a history or elevated enzymes | Pats with no liver history |
|---|---|---|
| Pats with liver pattern | TP 20 | FP 8 |
| Pats lacking liver pattern" | FN 49 | TN 173 |

Sensitivity=TP/(TP+FN)=20/69=0 29
Specificity = TN/(TN + FP) = 173/181 = 0 96
Predictive value=TP/(TP+FP)=20/28=0 71

agent tamoxiphene Two females with Crohn s disease and one with acute pleuropneumonia all taking oral contraceptives also had the pattern Two female outpatients 21 and 40 years of age using no oestrogens and with normal ceruloplasmin levels are included as is one man with orthopaedic disability but no clinical data concerning liver disease

## DISCUSSION

The results of this study demonstrate a characteristic fall in albumin levels with increasing inflammation and biopsy score This was expected and validates the histological classification system used Selective increases in levels of $\alpha_1$ AT compared to other acute phase reactants are clearly seen in nearly all diagnostic groups of liver disease The elevation in $\alpha_1$ AT levels corresponds well to total biopsy score and even more markedly to inflammatory activity indicating its value as an activity parameter Increased levels in liver disease may reflect decreased catabolism but more probably indicate an increased synthesis perhaps in response to local release of proteolytic enzymes from leukocytes and macrophages It is of interest to note that $\alpha_1$-AT levels are related to degree of hepatocellular necrosis inflammatory cell infiltration and Kupffer cell activity The elevations of $\alpha_1$ AT levels in alcoholic hepatitis are somewhat lower than those reported by other authors (9 12) This may be because blood samples in our study were taken during convalescence when liver biopsy was also feasible

Orosomucoid was nearly unchanged with all biopsy characteristics observed except for steatosis The significant increase in orosomucoid with increasing steatosis is difficult to interpret but may be related to ethanol induced damage

The frequent decrease in haptoglobin concentration contributes to the typical pattern of acute phase reactants in liver disease This decrease may result from portal hypertension with splenomegaly or increased boned marrow hemolysis due to ineffective erythropoiesis among other factors Interestingly the haptoglobin level was correlated to degree of fibrosis and/or cirrhosis (Table II) factors conducive to portal hypertension

The presence of the acute phase pattern typical for liver disease in some patients with oestrogen medication and no clinical or laboratory signs of liver disease invites speculation about the role of hyperoestrogenisation in the plasma protein pattern of liver disease Clinical signs of hyperoestrogenisation are common in chronic hepatocellular failure (8) and oestrogens are known to increase $\alpha_1$ AT levels (7) The normal ceruloplasmin levels in patients with the liver pattern contradict the assumption that hyperoestrogenisation is of importance in evoking this special pattern

The acute phase pattern for liver disease was found in 29% of unselected patients with a history of current acute or chronic liver disease The figure of 39% (48/124) among biopsy patients supports the observation of an increased extent of liver disease among patients with the pattern (Fig 3) The sensitivity of this typical pattern is nonetheless low This may be partly explained by the large number of patients with alcohol induced liver disease As indicated above the pattern in such disease is blurred by the relatively high orosomucoid level correlating to steatosis Despite its low sensitivity presence of the pattern in the absence of exogenous oestrogen is highly specific and predictive for liver disease

## ACKNOWLEDGEMENT

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## REFERENCES

1 Ferran G & Fabucci C Improved electroimmunodiffusion assay of fibrinogen Clin Chem 22 1390 1976
2 Jensen D M McFarlane I G Portmann B S Eddleston A L W F & Williams R Detection of antibodies directed against a liver specific membrane

lipoprotein in patients with acute and chronic hepatitis N Engl J Med 299 1 1978
3 Jeppsson J O Laurell C B & Fagerhol M Properties of isolated human alpha$_1$ antitrypsins of Pi types M S and Z Eur J Biochem 83 143 1978
4 Johansson B G Agarose gel electrophoresis Scand J Clin Lab Invest (Suppl) 124 7 1972
5 Kindmark C O & Laurell C B Sequential changes of the plasma protein pattern in innoculation hepatitis Scand J Clin Lab Invest (Suppl) 124 105 1972
6 Laurell C B Electroimmunoassay Scand J Clin Lab Invest (Suppl) 124 21 1972
7 Laurell C B Kullander S & Thorell J Effect of administration of a combined estrogen progestin contraceptive on the level of individual plasma proteins Scand J Clin Lab Invest 21 337 1967
8 Lester R Eagon P & Van Thiel D Feminization of the alcoholic the estrogen/testosterone ration (E/T) Gastroenterology 76 415 1979
9 Mihas A A Serum alpha$_1$ antitrypsin levels in alcoholic hepatitis Digestion 17(3) 275 1978
10 Scheuer P J Liver biopsy interpretation Balliere and Tindall London 1973
11 Sharp H L Bridges R A Krivit W & Freier E F Cirrhosis associated with alpha$_1$ antitrypsin deficiency A previously unrecognized inherited disorder J Lab Clin Med 73 934 1969
12 Skrede S Blomhoff J P Elgjo K & Gjone E Serum proteins in disease of the liver Scand J Clin Lab Invest 35 399 1975
13 Van Wals L & Lieber C Glutamate dehydrogenase a reliable marker of liver cell necrosis in the alcoholic Br Med J 2 1508 1977

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# The Effect of Renal Transplantation on Basal Serum Gastrin Concentration

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**ABSTRACT** Basal serum gastrin concentration was measured before and every week during the initial 5 weeks after renal transplantation in 9 of 20 patients with chronic renal failure who obtained a well functioning renal transplant Furthermore calcium and phosphorus metabolism in relation to serum gastrin was investigated in all 20 patients 5 weeks after transplantation Before renal transplantation serum gastrin was markedly elevated as compared with the levels in normal controls During the first 3–5 weeks after renal transplantation serum gastrin decreased towards normal values A slight but significant increase in serum gastrin persisted 5 weeks after transplantation No significant relation between changes in serum gastrin concentration and in calcium and phosphorus metabolism was observed

*Key words* serum gastrin chronic renal failure renal transplantation serum calcium

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Hypergastrinemia has been found both in non dialyzed and dialyzed patients with chronic renal failure and the increased serum gastrin concentrations have been explained mainly by a reduced renal degradation of the hormone (4 8) Hypergastrinemia has been suggested to be important for the pathogenesis of peptic ulceration in renal transplant patients (6)

As to our knowledge only one prospective study of serum gastrin concentration after renal transplantation has been reported (9) the present study was carried out to describe changes in basal serum gastrin concentration during the first 5 weeks after renal transplantation A significant correlation between the serum concentrations of calcium and gastrin has been observed in renal transplant patients (9) Thus calcium metabolism in relation to serum gastrin concentration was also investigated

## PATIENTS

The study comprised 20 patients 11 males and 9 females 18–64 years old (mean 45 4) One patient (no 4) had a radiologically proven peptic ulcer All the patients received a cadaver kidney which achieved a maximal creatinine clearance of 20–94 ml/min (mean 54) 1–5 weeks (mean 3–6) after transplantation The serum gastrin concentrations were compared with the values of 20 age and sex matched healthy controls

All kidney recipients received immunosuppressive therapy with prednisone and azathioprine The initial dose of prednisone was 2 mg/kg b wt During the first months this steroid dose was reduced to about 30 mg/day Rejection crises were treated with i v methylprednisolone in gram doses The usual daily dose of azathioprine was 2 mg/kg b wt

## METHODS

Serum concentrations of calcium (corrected for individual variation in protein binding) inorganic phosphate creatinine and gastrin were measured after an overnight fast in all patients 5 weeks after transplantation In 9 patients (Table I) these measurements were also done before and every week during the initial 5 weeks after renal transplantation

Serum concentrations of calcium phosphorus and creatinine were measured by autoanalyzer methods Cratinine clearance was measured using 24-hour urine collections

*Serum gastrin* was measured by a radioimmunoassay as described previously (2) The gastrin antiserum employed binds the four main components of gastrin in serum with equimolecular potency

*Statistical evaluations* Statistical differences between group means were determined by the Mann Whitney *U* test Correlation analysis was carried out by Spearman s

Table I *Clinical data on 9 of the patients*

CGN=chronic glomerulonephritis E=end stage kidney disease AN=analgesic nephropathy CPN=chronic pyelonephritis

| Pat no | Age (y) | Sex | Renal disease | Duration of dialysis (mo) |
|---|---|---|---|---|
| 1 | 58 | ♀ | CGN | 6 |
| 2 | 51 | ♀ | E | 15 |
| 3 | 57 | ♀ | AN | 1 |
| 4 | 47 | ♂ | CGN | 13 |
| 5 | 25 | ♂ | E | 14 |
| 6 | 28 | ♂ | CPN | 6 |
| 7 | 30 | ♂ | CGN | 12 |
| 8 | 54 | ♀ | CGN | 9 |
| 9 | 30 | ♂ | CGN | 14 |
| Mean | 42 2 | | | 10 0 |

rank correlation test The significance of changes in serum gastrin was tested with the Wilcoxon s rank sign test for paired comparisons

## PESULTS

*Serum gastrin during the initial 5 weeks after renal transplantation*

The mean serum gastrin concentration in the recipients was 148 pg/ml (range 58–470) at the time of transplantation compared with 28 pg/ml (range 10–55) in the healthy controls ($p<0.01$)

During the first 3–5 weeks after transplantation serum gastrin decreased significantly ($p=0.01$ $p=0.01$ $p=0.05$ respectively) (Fig 1) Patient 4 with radiologically proven peptic ulcer as well as the patients without peptic ulcer showed a marked fall in serum gastrin after transplantation

The normalization of serum gastrin followed the improvement of renal function (Fig 1) No significant correlation was however found between the mean values of serum gastrin and creatinine clearance ($R=-0.43$) between serum gastrin and serum calcium ($R=0.63$) or between serum gastrin and serum phosphorus ($R=0.79$ $p=0.05$ $n=7$) No relation between the dose of prednisone and serum gastrin was observed None of these patients developed gastrointestinal complications after transplantation

*Serum gastrin 5 weeks after renal transplantation*

The mean serum gastrin concentration was 53 pg/ml (range 17–114) in the renal transplant patients (Table II) compared with 28 pg/ml (range 10–55) in the healthy controls ($p<0.01$) No significant relation was found between serum gastrin and creatinine clearance ($R=-0.03$) between serum gastrin and serum calcium ($R=0.28$) or between serum gastrin and serum phosphorus ($R=0.20$)

## DISCUSSION

The present study showed as previously found (4 7 8) high basal serum gastrin concentrations in patients with chronic renal failure Previous studies of serum gastrin in renal transplant patients have been conflicting describing elevated (6) or normal (9) serum concentration after renal transplantation In renal transplant patients studied 0 5–7 years after transplantation Collins et al (6) found serum gastrin concentrations at the same level as in patients with chronic renal failure and concluded that the high serum gastrin concentration might be important in the pathogenesis of peptic ulceration The present study could however not support this finding as serum gastrin values decreased towards normal during the initial 3 weeks after renal transplantation following the normalization of kidney function although a significantly increased serum

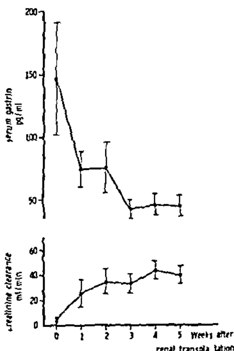



*Fig 1* Serum gastrin and creatinine clearance (mean ± S E M ) in 9 renal transplant recipients before and during the initial 5 weeks after transplantation

Table II *S gastrin s calcium s phosphorus and creatinine clearance in 20 renal transplant recipients 5 weeks after transplantation*

| | S-gastrin (pg/ml) | S-calcium (mg/100 ml) | S-phosphorus (mg/100 ml) | Creatinine clearance (ml/min) |
|---|---|---|---|---|
| Mean | 53 | 9 8 | 2 7 | 53 6 |
| Range | 17–114 | 8 9–11 3 | 1 7–4 5 | 20–96 |
| Normal range | 0–90 | 9 0–10 6 | 2 7–4 5 | 70–130 |

gastrin concentration compared with normal controls was still seen 5 weeks after the transplantation Muolo et al (9) found in agreement with this finding a rapid fall in serum gastrin concentration in one renal transplant patient investigated Furthermore these authors found normal serum gastrin concentration in 24 recipients studied 6–60 months after transplantation

The marked fall in serum gastrin during the initial 3 weeks after renal transplantation seems to indicate that increased serum gastrin in patients with chronic renal failure is closely associated with reduced glomerular filtration rate The increased serum gastrin concentration may be explained by a reduced degradation of the hormone in the kidneys as the kidney is the primary organ of catabolism of low molecular proteins (11) In agreement with this a lower metabolic clearance rate of polypeptide hormones such as calcitonin (1) and insulin (9) has been reported in patients with chronic renal failure The persistently slight but significantly elevated serum gastrin concentration measured 5 weeks after renal transplantation may be due to a slightly reduced renal function (mean creatinine clearance of 53 ml/min)

Calcium infusion may stimulate gastrin secretion in man (5) and higher basal serum gastrin concentrations than in control patients have previously been found in hemodialyzed patients treated with 1α hydroxyvitamin $D_3$ inducing a mean serum calcium rise of 1 0 mg/100 ml (3) Furthermore Muolo et al (9) found a significant positive relation between serum gastrin and serum calcium in renal transplant patients studied 6–60 months after transplantation We could however not demonstrate such a correlation in renal transplant patients studied 5 weeks after transplantation The reason for the different findings in these two studied is not known but some of the discrepancy may be due to the different intervals between transplantation and study

## ACKNOWLEDGEMENTS

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## REFERENCES

1 Ardaillou R Sizonenko P Meyrier A Vallee G & Beaugas C Metabolic clearance rate of radioiodinated human calcitonin in man J Clin Invest 49 2345 1970
2 Brandsborg O Brandsborg M & Christensen N J Plasma adrenaline and serum gastrin studies in insulin induced hypoglycemia and after adrenaline infusions Gastroenterology 68 455 1975
3 Christensen C K Nielsen H E Brandsborg M & Brandsborg O Increased serum gastrin concentration in hemodialysis patients treated with 1 alpha hydroxy vitamin $D_3$ Scand J Gastroenterol 12 967 1977
4 Christensen C K Nielsen H E Kamstrup O Olsen K J Brandsborg M & Brandsborg O Serum gastrin and serum calcitonin in patients with chronic renal failure Acta Endocrinol 91 564 1979
5 Christiansen J Rehfeld J F & Stadil F Interaction of calcium and magnesium on gastric acid secretion and serum gastrin concentration in man Gastroenterology 68 1140 1975
6 Collins S M Barnes C C & Track N S Gastrointestinal hormones in chronic renal failure and renal transplant recipients Scand J Gastroenterol (Suppl) 13 42 1978
7 Gedde Dahl D Serum gastrin response to food stimulation in male uraemic patients Scand J Gastroenterol 10 683 1975
8 Korman M G Laver M C & Hansky J Hypergastrinaemia in chronic renal failure Br Med J 1 209 1972
9 Muolo A Ghidini O Tonon M Galvani E Baratta P F & Confortini P Serum gastrin levels in patients with renal failure on maintenance haemodialysis and after successful kidney transplantation Ric Clin Lab 6 277 1976
10 Silvers A Svenson R S Farquhar J W & Reaven G M Derivation of a three compartment model describing disappearance of plasma insulin 131 I in man J Clin Invest 48 1461 1969
11 Strober W & Waldmann T A The role of the kidney in the metabolism of plasma proteins Proc 6th Int Congr Nephrol 1975 pp 392–405 Karger Basel 1976

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PRELIMINARY REPORT

# Ultra-Microcrystals in Pyrophosphate Arthropathy

*Crystal Identification and Case Report*

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**ABSTRACT A patient with pyrophosphate arthropathy is reported who had no calcifications on joint radiographs, and no crystals were found in polarized light microscopy of the synovial fluid Using techniques for identification of crystals at the ultrastructural level abundant small (≤1μ) pyrophosphate crystals were recognized and identified The possibility of "ultramicrocrystal depositions ', including pyrophosphate arthropathy, is important to consider in acute arthritides since small crystals might cause more intense inflammation and be the cause of arthritides, hitherto not possible to classify**

*Key words* pyrophosphate microcrystal arthritis pseudo gout chondrocalcinosis

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The use of polarized light microscopy for the identification of synovial fluid microcrystals (17) has been of great diagnostic value in the rheumatological routine Thus pyrophosphate arthropathy has become a frequent clinical experience and has been found to include a considerable variation of clinical syndromes (4 5 16) although it was recognized only 20 years ago (18 21)

Rhomboid crystals of 20 μ and with relatively strong positive birefringence are often reported as the typical finding in pyrophosphate arthropathy The frequent occurrence of non rhomboid small crystals with weak birefringence and intracellular location however makes crystal screening and analysis in the polarized light difficult New techniques (7) have enabled recognition and more precise identification of mineral particles in synovial fluids (1 9 10 12)

A case of pyrophosphate arthropathy with negative findings in radiographs of joints and with no crystals observed in polarized light microscopy is presented Crystals of pyrophosphate were easily recognized and identified on the ultra structural level by applying new diagnostic routines

## CASE REPORT

A 65 year-old man on disablement pension for two years due to osteoarthrosis of his left knee joint No calcifications were observed in the cartilage or in the menisci on the radiographs He was referred to the Department of Rheumatology because of acute arthritis in his right elbow joint and fever 13 cm³ of a somewhat opaque synovial fluid were obtained at a joint tap The fluid contained 30000 white cells/mm³ 82 % of which were granulocytes No crystals were found in polarized light microscopy of wet preparations No bacteria were found after the staining of synovial fluid smears or in cultures of fluids

ESR was moderately elevated (20 mm/h) and acute phase reactants were slightly above normal levels Other routine tests were normal and no cartilage calcifications were revealed on radiographs The patient responded to treatment with indomethacin Because apatite deposition was suspected the synovial fluid granulocytes were prepared for electron microscope examination and electron probe microanalysis

## *MATERIAL AND METHODS*

Methods for crystal identification of synovial fluids are summarized in Fig 1 Thus fresh synovial fluid was examined immediately after the joint tap as a wet preparation in a Zeiss Photomicroscope II specially equipped for polarizing microscopy with rotating polarizer and analyser lambda plate rotating stage and quartz halogen light source Another sample of the synovial fluid was diluted with saline and centrifuged in a Shandon cytospin centrifuge for 10 min at 600 rpm directly on to coated EM grids The specimens were examined in a Jeol 100 CX electron microscope with scanning equipment ASID-4 and with a Kevex 5100 X ray energy dispersive spectrometer system attached

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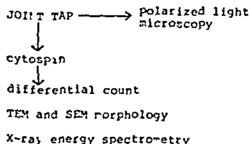



*Fig 1* Scheme for the routine procedures for identification of crystal material in synovial fluids After joint tap fresh wet preparations are screened by polariscopy Another portion is diluted cytospinned for differential count staining and deposited on EM grids for transmission and scanning (TEM and SEM respectively) morphology and X ray energy spectrometry

## RESULTS

Even though optimal equipment for polarized light microscopy was used for the identification of synovial fluid microcrystals no crystalline material was found in this case After cytospin of the fresh synovial fluid granulocytes directly on to EM grids without fixation or other treatment typical pyrophosphate crystals were recognized at high magnification in the electron microscope (Fig 2) The crystals were located in the granulocytes and were highly sensitive to the electron beam which gave them a bubbled' appearance (Fig 2)

Great variations in crystal size and shape were observed Only a few crystals had a length of 1 μ the majority being smaller than 0 5 μ Crystals of different sizes were often found adjacent to each other (Fig 2) and were frequently packed as small clusters with irregular outlines (Fig 3)

In X ray energy dispersive spectrometry the crystals were shown to contain phosphate and calcium (Fig 4) in a proportion equivalent to the finding in control samples of pure crystal powder of calcium pyrophosphate dihydrate identified by X ray diffraction The ratio phosphate/calcium in these powders is 0 9 as is found in the crystals of the present case

## DISCUSSION

Methods for identification of synovial fluid crystals are as follows At macroscopial level powder X ray diffraction infrared spectrometry At light microscopical level polarized light microscopy micro-X ray diffraction At ultrastructural levels electron diffraction electron probe microanalysis (X ray energy dispersive spectrometry and X ray energy wave length spectrometry) Powder X ray diffraction is feasible when synovial fluids contain numerous crystals as originally described (18) Infrared spectrometry has been used for smaller amounts of crystals (7 10) and differentiates between pyrophosphate and apatite Polarized light microscopy is of great value in the clinical routine

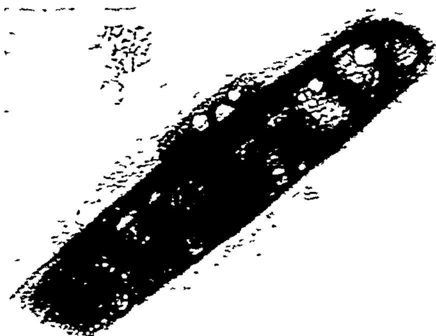


*Fig 2* Ultra microphotograph of an intracellular crystal of calcium pyrophosphate in the synovial fluid This crystal is 0 4 μ long Note the small crystal adjacent to the large one and the "bubbling" appearance under the electron beam ×255 000



*Fig 3* Ultra microphotograph of a small cluster of crystals giving an irregular appearance to the outlines and possibly also interfering with the properties of birefringence of this particle ×45000

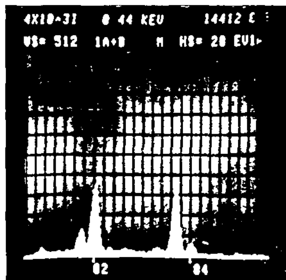



*Fig 4* Electron probe analysis energy dispersive spectrometry of the crystal in Fig 2 Equal values are obtained for phosphorus and calcium which is compatible with calcium pyrophosphate dihydrate *but is also found in* orthophosphates

since it is sufficient for the identification of urate and pyrophosphate in most instances (7 16) Micro-X ray diffraction is applicable for finger print identification of small amounts of crystalline materials as in microscopic sections (2 3)

To visualize crystals under the size of 1–2 μ and for the analysis of single microcrystals EM techniques must be utilized Transmission EM has demonstrated a great variation in sizes and shapes of pyrophosphate crystals in cartilage (6) compatible with the finding of microcrystals in the synovial fluid However the difficulties of identifying crystal shape and size when observed in two planes only are illustrated in the scanning EM of pyrophosphate crystals of a synovial fluid from another patient with the usual finding of abundant large (10–20 μ) crystals (Fig 5) The cystospin technique described in this paper allows rapid preparation of synovial fluids for EM

The crystals may have typical outlines (Fig 2) but crystallographic identification of single crystals is essential in order to rule out artefacts and depositions of several kinds of crystals Electron diffraction is not feasible on pyrophosphates due to the high sensitivity of the crystal to the energy of the electron beam (Figs 2 and 3) This necessitates the use of electron probe microanalysis for which the X ray energy dispersive system utilized in the present investigation is the most suitable for routine screening It does not allow differentiation between pyrophosphates and orthophosphates for which an X ray energy wavelength system is required (12) That system is more commonly found in SEM and is thus not applicable to intracellular crystals It is considerably more laborious but can be added to the present method when adequate samples are available

Negative crystal findings in routine polarizing microscopy of initial joint taps is a common experience in gout (13 17 19 20) The lack of synovial fluid crystals in patients with joint calcifications has been difficult to evaluate due to the high frequency of asymptomatic chondrocalcinosis (4) The poor correlation between the number of crystals and the intensity of the inflammation may be due to crystal structure (15) or interaction with other components of the joint fluid in the individual (14) The possibility of an initial phase of precipitation of crystal sizes beyond the resolution of the light microscope particularly when considering the influence of the refractile properties of synovial fluid on the bire



*Fig 5* Scanning ultra micrograph of a synovial fluid sample from another case of pyrophosphate arthropathy illustrating the usual finding of abundant large (10–20 $\mu$) crystals Note however the variable shapes and sizes of the pyrophosphate crystals and the varying direction of crystal orientation in the specimen ×1 180

fringence of the crystal (13 20) cannot be ruled out The importance of crystal size is supported by the finding of an inverse correlation between intensity of inflammation and crystal size in experimental systems (11) Ultra microcrystals including pyrophosphate as illustrated by the present case might be the cause of hitherto unclassified acute arthritides in a number of patients

## ACKNOWLEDGMENTS

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## REFERENCES

1 Amor B Cherot A & Delbarre F La rhumatisme à hydroxyapatite (La maladie des calcifications tendineuses multiple) I Etude clinique Rev Rhum 44 301 1977
2 Bjelle A & Sundstrom B Micro X ray diffraction of cartilage biopsy specimens in articular chondrocalcinosis Acta Pathol Microbiol Scand 76 497 1969
3 — A morphological study of articular cartilage in pyrophosphate arthropathy Ann Rhem Dis 31 449 1972
4 — Pyrophosphate arthropathy Scand J Rheumatol 8 145 1979
5 Bjelle A & Sundén G Pyrophosphate arthropathy A clinical study of fifty cases J Bone Joint Surg (B) 56 246 1974
6 Bjelle A & Sundstrom B K G An ultrastructural study of the articular cartilage in calcium pyrophosphate dihydrate (CCPD) crystal deposition disease (chondrocalcinosis articularis) Calcif Tissue Res 19 63 1975
7 Crocker P Dieppe P A Tyler G Chapman S K & Willoughby D A The identification of particulate matter in biological tissues and fluids J Pathol 121 37 1977
8 Dieppe P A Crystal induced inflammation and osteoarthritis In Perspectives in inflammation (ed D A Willoughby et al) p 225 MTP Press Lancaster 1977
9 Dieppe P A Huskinsson E C Crocker P & Willoughby D A Apatite deposition disease A new arthropathy Lancet 1 266 1976
10 Doyle D V Dieppe P A Crocker P R Ibe K & Willoughby D A Mixed crystal deposition in an osteoarthritic joint J Pathol 123 1 1977
11 Dunn C J Doyle D V & Willoughby D A Experimental methods in the study of crystal deposition diseases Eur J Rheumatol Inflamm 1 135 1978
12 Faure G Netter P Malaman B & Steinmetz J Monocrystalline calcium hydrogen phosphate dihydrate in destructive arthropathies of chondrocalcinosis Lancet 1 142 1977
13 Honig S Gorevic P Hoffstein S & Weissman G Crystal deposition disease Am J Med 63 161 1977
14 Kozin F & McCarthy D J Protein adsorption to monosodium urate calcium pyrophosphate dihydrate and silica crystals Arthritis Rheum 19 433 1976
15 Mandel N S The structural basis of crystal induced membranolysis Arthritis Rheum 19 439 1976
16 McCarty D J Jr Calcium phyrophosphate dihydrate crystal deposition disease Arthritis Rheum 19 275 1976
17 McCarty D J & Hollander J L Identification of urate in gouty synovial fluid Ann Intern Med 54 452 1961
18 McCarty D J Jr Kohn N N & Faires J S The significance of calcium phosphate crystals in the synovial fluid of arthritic patients The Pseudogout syndrome I Clinical aspects Ann Intern Med 56 711 1962
19 Romanoff N R Rubinow A Canoso J J & Spark E C Gout without crystals on initial synovial fluid analysis Postgrad Med J 54 95 1978
20 Schumacher R H Jimenez S A Gibson T Pascual E Traycoff R Dorwart B B & Reginato A J Acute gouty arthritis without urate crystals identified on initial examination of synovial fluid Arthritis Rheum 18 603 1975
21 Zitnan D & Sitaj S Mnohopocetna familiarlu kalcificaciz articularynch chrupiek Bratisl Lek Listy 28 217 1959

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# Penicillamine Treatment in Rheumatoid Arthritis

## A Retrospective Study

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ABSTRACT Out of 64 patients with rheumatoid arthritis (RA), 42 were treated with D-penicillamine (D-Pa) for more than 6 months and 22 for less than 6 months The latter patients were excluded from the evaluation of the effect The former patients were treated with doses of 600–1 250 mg daily for 6–41 months (mean 16 8) The clinical effect was retrospectively assessed as favourable in 24 patients, 12 did not respond and the effect could not be assessed in 6 The clinical assessment was supported by significant reductions of ESR and orosomucoid Adverse reactions although rarely serious led to withdrawal of the drug in 25 (39 %) of the 64 patients It is concluded that D-Pa is a valuable drug in the treatment of severe RA

*Key words* penicillamine rheumatoid arthritis

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It has been established in double blind investigations that D-penicillamine (D-Pa) is effective in the treatment of rheumatoid arthritis (RA) (7 14 15) It is also well known that the drug is frequently associated with adverse reactions (10 14) Only a few long term studies have demonstrated the benefit of the drug (6 10) Therefore we have performed a retrospective study on 64 RA patients treated with D-Pa in our clinic from May 1973 to Aug 1978

## PATIENTS AND METHODS

Of the 64 patients 50 fullfilled the ARA criteria (2) for classical and 14 for definite RA The age of the patients and the duration of the disease are given in Table I The functional handicap of the patients appears from Table II Compared with the results of Allander s survey (1) which can be regarded as representative for a Swedish RA population our patients are more severely handicapped Our patients also show severe joint destruction according to the ARA anatomical stage criteria (16) (Table III) There were no differences in age sex duration of disease functional handicap or joint destruction between responders and non responders

The following extra articular manifestations of RA were noticed subcutaneous nodules in 23 patients vasculitis in 9 Sjogren s syndrome in 3 pleuritis in 3 and episcleritis in 1 patient

The therapy previous to D-Pa appears from Table IV Only one patient had not been treated with any of the drugs mentioned in the table There was no significant difference in previous treatment between responders and non responders

The principles for D-Pa therapy at our department are given in Table V

Statistical analysis was performed as the difference between means using Student s *t* test (18)

### *Evaluation of the effect*

Since treatment for less than 6 months is considered inadequate for estimating results of therapy (12) and since placebo effects might be induced by a change of therapy 22 patients treated for less than 6 months were excluded from the evaluation of the effect Of these patients 5 had started D-Pa therapy after Feb 1978 and 17 had discontinued The remaining 42 patients were treated for 6–41 months (mean 16 8)

The effect of the drug was assessed using the following clinical criteria 1) Patient s opinion concerning relief of pain and morning stiffness and improvement of joint mobility and general well being 2) The treating physician s assessment expressed either as a clear opinion of improvement or as a reduction by 50 % or more of the number of joints with active synovitis 3) Reduction of concomitant steroid therapy by 25 % or more When two of these three criteria were met therapy was considered effective

## RESULTS

### *Effect of the drug*

Of the 42 patients who were treated for 6 months or more 24 responded to the drug 12 did not respond

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*Abbreviations* RA = rheumatoid arthritis D-Pa = D-penicillamine ANA = antinuclear antibody

Table I *Age and sex distribution of the patients and duration of the disease*

| | N | Mean | Range |
|---|---|---|---|
| Age (y) | | | |
| Women | 46 | 54 9 | 27–75 |
| Men | 18 | 57 6 | 34–71 |
| Duration of disease (y) | | | |
| Women | 46 | 11 6 | 1–38 |
| Men | 18 | 11 5 | 1–30 |

Table II *Functional handicap*

| | Present study | | | | |
|---|---|---|---|---|---|
| Functional class* | Total (N=64) n | % | Responders (N=24) (%) | Non responders (N=12) (%) | Allander's study (1) (%) |
| I | | | | | 4 |
| II | 37 | 58 | 67 | 67 | 83 |
| III | 23 | 36 | 29 | 25 | 11 |
| IV | 4 | 6 | 4 | 8 | 2 |

* As defined by Steinbrocker et al (16)

Table III *Anatomical stage*

| Anatomical stage* | Total (N=63) n | % | Responders (N=23) (%) | Non responders (N=12) (%) |
|---|---|---|---|---|
| I | 1 | 2 | 4 | |
| II | 31 | 51 | 52 | 58 |
| III | 27 | 44 | 39 | 42 |
| IV | 2 | 3 | 4 | |

* As defined by Steinbrocker et al (16)

and the effect could not be determined in 6 The concomitant steroid therapy was reduced from 7 1 to 4 2 mg prednisolone ($p<0.01$) per day (mean values) for the responders and from 6 8 to 6 0 mg (N S ) for the non responders Our clinical assessment was supported by significant reductions of ESR ($p<0.05$) and p-orosomucoid ($p<0.001$) in the responders but not in the non responders (Table VI)

There was no statistical difference between responders and non responders in these respects before treatment

Table IV *Previous treatment*

| | Total (N=64) (%) | Responders (N=24) (%) | Non responders (N=12) (%) |
|---|---|---|---|
| Chloroquine | 89 | 92 | 83 |
| Prednisolone | 73 | 79 | 67 |
| ACTH | 9 | 0 | 17 |
| Gold salts | 36 | 38 | 25 |
| Cytostatics | 31 | 42 | 17 |

Table V *Dosage principles*

| | Initial dose (mg) | Increment (mg) | Max dose (mg) |
|---|---|---|---|
| Before 1976 | 150–300 | 150–300 every 2nd–4th week | 750–1 250 |
| From 1976 onwards | 150–250 | 150–250 every 4th week | 750 |

Table VI *Laboratory findings before (B) and at the end of treatment (A) (mean values)*

| | Responders (N=24) | | Non responders (N=12) | |
|---|---|---|---|---|
| Hb (g/l) | | | | |
| B | 119 | N S | 112 | N S |
| A | 127 | | 109 | |
| ESR (mm) | | | | |
| B | 57 | $p<0.05$ | 74 | N S |
| A | 37 | | 47 | |
| p-Orosomucoid (g/l) | (N=16) | | (N=9) | |
| B | 2 09 | $p<0.001$ | 2 12 | N S |
| A | 1 55 | | 2 29 | |

*Adverse reactions*

A total number of 66 adverse reactions were noted in 46 of the 64 patients (Table VII) leading to withdrawal of the drug in 25 The adverse reactions appeared as frequently before as after 6 months treatment There were few serious reactions e g thrombocytopenia below $100\times10^9$ in 3 patients leucopenia ($1.8\times10^9$) in one patient and albuminuria of more than 200 mg/l in 3 patients Increasing antinuclear antibody (ANA) titers were recorded in 2 patients and increasing levels of serum transferases in 6 leading to withdrawal in 3

Table VII *Adverse reactions*

| | Total no | Treated for <6 mo: Total no | No of withdrawals | Treated for >6 mo: Total no | No of withdrawals |
|---|---|---|---|---|---|
| Thrombocytopenia <100×10⁹/l | 3 | 1 | 1 | 2 | 1 |
| Leucopenia <2 7×10⁹/l | 3 | 1 | 1 | 2 | 1 |
| Eosinophilia >8% | 4 | 2 | 1 | 2 | 1 |
| Albuminuria | | | | | |
| >200 mg/l | 3 | 1 | 1 | 2 | 1 |
| <200 mg/l | 8 | 4 | 2 | 4 | 1 |
| Haematuria | 1 | 1 | 1 | 0 | 0 |
| Raised serum transferases | 6 | 1 | 1 | 5 | 2 |
| Increased ANA titer | 2 | 1 | 1 | 1 | 1 |
| Oral ulcers sore mouth | 3 | 1 | 1 | 2 | 1 |
| Exanthema pruritus | 13 | 4 | 3 | 9 | 0 |
| Urticaria | 1 | 0 | 0 | 1 | 1 |
| Localised angioedema | 1 | 1 | 0 | 0 | 0 |
| Pityriasis rosea | 2 | 1 | 0 | 1 | 0 |
| Pergament skin | 1 | 1 | 1 | 0 | 0 |
| Loss of hair | 1 | 1 | 0 | 0 | 0 |
| Loss of taste | 5 | 2 | 1 | 3 | 0 |
| Nausea | 5 | 1 | 0 | 4 | 0 |
| Diarrhoea | 3 | 1 | 0 | 2 | 0 |
| Amenorrhoea | 1 | 0 | 0 | 1 | 0 |

## DISCUSSION

This study deals with severely ill RA patients treated earlier with active and potentially dangerous drugs The results indicate that at least 55% (24 of 42) of patients treated for 6 months or longer respond to D-Pa The results are in accordance with those of other authors (6 10 14)

Adverse reactions led to withdrawal of the drug in 25 (39%) of the 64 patients Previous investigators have noted a somewhat lower frequency of withdrawals (Table VIII)

Signs of renal affection have been present in 12 patients only 3 of whom have excreted more albumin than 200 mg/l The albuminuria disappeared completely in all but one patient who is suffering from amyloidosis Bacon et al (3) have established that proteinuria in the absence of nephrotic syndrome can remain for 2 years after withdrawal of D-Pa They also found evidence of basal membrane immune complexes in D-Pa treated patients with pure proteinuria seemingly of the same type as in those with complete nephrotic syndrome From these findings they conclude that there are no qualitative differences between renal affection in nephrotic syndrome and in pure proteinuria As a consequence we have decided to withdraw D-Pa already *when albuminuria exceeds 200 mg/l* Similar principles are applied by Davis and Bleehen (5)

Furthermore it has recently been found that continuation of D-Pa administration despite proteinuria can lead to a Goodpasture like syndrome (8) On the other hand Hill (10) has described 4 patients with albuminuria exceeding 10 g/24 hours whose *renal function recovered completely within 2 years* after withdrawal of D-Pa Huskisson (11) does not consider it necessary to stop treatment until the albuminuria exceeds 5 g/24 hours unless the patient becomes nephrotic

Positive ANA and SLE like syndromes have been noted as adverse reactions to D-Pa (4 9) We have observed only 2 cases with increasing ANA but without clinical evidence of SLE

Increasing levels of serum transferases have been

Table VIII *Adverse reactions leading to withdrawal of D-Pa treatment*

| Author | Total no | Withdrawals n | % |
|---|---|---|---|
| Multicentre Trial Group (14) | 52 | 16 | 31 |
| Day et al (6) | 85 | 22 | 25 |
| Dixon et al (7) | 78 | 27 | 35 |
| Hill (10) | 108 | 35 | 32 |
| Shiokawa et al (15) | 90 | 18 | 20 |
| Makisara et al (13) | 50 | 13 | 26 |
| Present study | 64 | 25 | 39 |

reported (13 17 19) Serum transferases in all of our 6 patients with raised levels have normalized and 3 of them have been able to continue the treatment

Contrary to others (7) no connection was established between frequency of adverse reactions and the dosage of D-Pa probably due to the fact that few patients received more than 1 000 mg D-Pa daily

To summarize we believe that the data presented here although retrospective allow us to conclude that D-Pa is a valuable drug in the treatment of severe RA In our opinion it should be used on the same indications as gold salts i e before administration of cytostatic drugs

## REFERENCES


1 Allander E A population survey of rheumatoid arthritis Acta Rheumatol Scand (Suppl) 15 1970
2 American Rheumatism Association Diagnostic criteria for rheumatoid arthritis Ann Rheum Dis 18 49 1959
3 Bacon P A Tribe C R Mackenzie J C Verrier Jones J Cumming R H & Amer B Penicillamine nephropathy in rheumatoid arthritis Q J Med 180 661 1976
4 Crouzet J Camus J P Leca A P Guillien P & Lievre J A Lupus induit par la d penicillamine au cours du traitement de la polyarthrite rheumatoide Ann Med Interne (Paris) 125 71 1974
5 Davis P & Bleehen S S D-penicillamine in the treatment of rheumatoid arthritis and progressive systemic sclerosis Br J Dermatol 94 70 1976
6 Day A T Golding J R Lee P N & Butterworth A Penicillamine in rheumatoid disease A long term study Br Med J 1 180 1974
7 Dixon A St J Davies J Dormandy T L Hamilton E B D Holt P J L Mason R M Thomson M Weber J C D & Zutshi D W Synthetic d penicillamine in rheumatoid arthritis Ann Rheum Dis 34 416 1975
8 Editorial Renal complications of connective tissue disease Br Med J 2 1517 1978
9 Harpey J P Lupus like syndromes induced by drugs Ann Allergy 33 256 1974
10 Hill H F H Treatment of rheumatoid arthritis with penicillamine Semin Arthritis Rheum 6 361 1977
11 Huskisson E C In Drug treatment of the rheumatic diseases (ed D Hart) p 49 ADIS Press London 1978
12 Jaffe I D-penicillamine Bull Rheum Dis 28 948 1978
13 Makisara P Nissila M Kajander A Martio J von Essen R Anttila P & Makisara G L Comparison of penicillamine and gold treatment in early rheumatoid arthritis Scand J Rheumatol 7 166 1978
14 Multicentre Trial Group Controlled trial of d penicillamine in severe rheumatoid arthritis Lancet 2 275 1973
15 Shiokawa Y Horiuchi Y Honma M Kageyama T Okada T & Azuma T Clinical evaluation of d penicillamine by multicentric double blind comparative study in chronic rheumatoid arthritis Arthritis Rheum 20 1464 1977
16 Steinbrocker O Traeger C & Batterman R Therapeutic criteria in rheumatoid arthritis JAMA 140 659 1949
17 Strandberg O Penicillamine treatment of rheumatic disease in an out patient unit In Proceedings of the 16th Scandinavian Rheumatology Congress p 36 1976
18 Swinscow T D V Statistics at square one p 25 BMA London 1978
19 Wollheim F Henningsen N C & Sjöblom K G Penicillamin inducerad leverskada hos två patienter med rheumatoid artrit Sv Läkaresällsk Handl 85 455 1976

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# Clinical Significance of Mitogen-Induced Responses in Lymphocytes from Patients with Chronic Lymphocytic Leukemia

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ABSTRACT The mitogenic response patterns as well as surface membrane receptors of peripheral blood lymphocytes were investigated repeatedly during progression of the disease in 27 patients with chronic lymphocytic leukemia Each patient was characterized by a reproducible mitogenic response pattern Eleven patients who required treatment within 0–24 months after diagnosis had significantly higher cellular responses to dextran sulphate (DxS) and lipopolysaccharide (LPS) than 10 patients who have not required treatment within an observation time of 10–40 months from diagnosis The high LPS and DxS responses which may indicate leukemias composed of more immature cells, appear to predict a poor prognosis

*Key words* CLL mitogens prognosis differentiation
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Chronic lymphocytic leukemia (CLL) generally denotes a slow and moderate progress of clinical symptoms and the median survival time from diagnosis is 4–6 years (1 6 9 16) However some patients exhibit a more aggressive course of the disease with fatal complications developing within months after diagnosis (16) Since the symptoms are generally mild at onset of the disease it is likely that some of these patients have had a subclinical form of the disease for a long time before diagnosis This makes it difficult to evaluate prognostic parameters of the disease as exemplified in the study by Rai et al (16) who presented a staging system describing the estimated survival after the development of certain symptoms Patients in stages III and IV were characterized by anemia and thrombocytopenia and the median survival time of patients in these stages was 19 months as compared to 150 months in stage 0 i e patients with blood and bone marrow lymphocytosis only

The small CLL lymphocytes show a pronounced morphological homogeneity in different patients It is therefore tempting to speculate that the disease is a result of genetically identical events leading to tumor transformation of one and the same subgroup of lymphocytes in different patients A similar clinical course for most patients would follow and all the aggressive cases would simply reflect late diagnosis However clinical complications are not always parallelled by a continuous expansion of the leukemic cell population Thus lymphocytosis splenomegaly and lymph node enlargement are not always related to the development of thrombocytopenia and anemia This rather speaks for the existence of subgroups of CLL characterized by different properties of the CLL cells

Various investigations of cellular properties have been undertaken in attempts to further classify CLL cells Recently both the HP receptor (helix pomatia) (8) and surface membrane immunoglobulins (13) have been shown to be of value for demonstrating cellular differences of clinical importance For example patients with CLL cells which express surface Ig kappa light chains have a more benign disease than those whose cells express Ig lambda light chains

In earlier reports (17 18 19 20) we have presented evidence of a functional heterogeneity of the

*Abbreviations* CLL = chronic lymphocytic leukemia PBA = polyclonal B cell activator PHA = phytohemagglutinin WBC = white blood cell count DxS = dextran sulphate LPS = lipopolysaccharide PPD = purified protein derivative BSS = balanced salt solution E RFC = E rosette forming cells T = treated NT = non treated Hb = hemoglobin Tc = thrombocyte

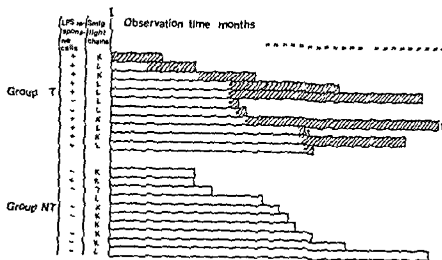



*Fig 1* Distribution of 10 patients not requiring treatment during the observation period (*group NT*) and 11 patients requiring treatment (*group T*) regarding SmIg kappa and lambda light chain restriction and LPS responses in peripheral blood lymphocytes Bars = total observation period from first investigation + − = LPS response of peripheral blood lymphocytes ● = start of treatment † = time of death

leukemic cells from different patients Thus there is a diversity of CLL cell in vitro responses to stimulation by mitogens with known polyclonal B cell activating (PBA) properties Individual lymphocyte cultures demonstrate a distinct response pattern following stimulation with different activating substances These patterns are influenced by special properties of the leukemic cells most probably the presence on the cell surface of different receptors for PBA substances Such receptors occur on cells at various stages of maturation and therefore suggest differences in cellular differentiation of responding cells (7) Also the presence of accessory cells such as T cells with helper effects on the CLL cell responses can influence these response patterns (20) Furthermore, T cell mitogens such as phytohemagglutinin (PHA) have been demonstrated to indirectly stimulate CLL cells (17 20) and have therefore been included in our studies

In the present study the mitogenic patterns are defined at diagnosis and during the course of the disease in order to evaluate their clinical importance and value for prediction of the prognosis Since the development of thrombocytopenia and anemia (16) as well as the surface Ig kappa or lambda light chain restriction (13) have previously been shown to contain prognostic information these parameters are also included in the study

## MATERIALS AND METHODS

*Cells* Lymphocyte suspensions were prepared from heparinized venous blood by flotation on a Ficoll Isopaque gradient (2)

*Patients* All 27 patients with newly diagnosed CLL admitted to the Section of Oncology and Hematology at Huddinge Hospital have been investigated during an observation period of 3 years Only one patient required treatment upon referral to hospital due to severe anemia The others had either none or only moderate symptoms such as slight lymph node enlargements or splenomegaly which did not require treatment at the time of diagnosis All patients had increased white blood cell counts (WBC) and a dominance of typical small lymphocytes in the peripheral blood and bone marrow Peripheral blood cell counts varied between 16 3 and 129 0×10⁹/l and the relative number of T cells determined as E RFC% (see below) varied between 0 5 and 24% Treatment was initiated according to an agreed protocol adopted by several hospitals in the Stockholm Uppsala and Orebro regions (10) Accordingly treatment with chlorambucil and prednisolone or prednimustine was given when required because of anemia thrombocytopenia spleen liver or lymph node enlargement or generalised symptoms such as severe fatigue All investigations were performed while the patients were still untreated in most cases at the time of diagnosis (I) and about 6 months thereafter (II)

During the whole observation period 11 patients have required treatment Ten other patients who have been observed for more than 10 months have not required treatment These patients are here referred to as the T (treated) and NT (non treated) groups

*Culture procedure and assay for DNA synthesis* As described in detail earlier (19) microcultures of lymphocytes were stimulated by three different concentrations of the mitogens PHA (diluted 1/1000 1/100 1/10) dextran sulphate (DxS) (5 50 500 μg/ml) lipopolysaccharide (LPS) (2 5 25 100 μg/ml) anti $\beta_2$ m (diluted 1/40 1/10 1/4) and purified protein derivative (PPD) (2 5 25 250 μg/ml) Cultures were harvested on day 3 and assayed for increased DNA synthesis by ³H thymidine incorporation added 24 hours prior to harvest

*Assay for B cells* Isolated lymphocytes were incubated in balanced salt solution (BSS) for 30 min at 37°C After three washings in 37°C BSS the cells were incubated with fluorescein conjugated F(ab₂) fragments of goat antihuman

kappa and lambda sera and thereafter washed three times in ice cold BSS (Kemila Preparat Kallestad Minnesota) (15) *The cells were then examined for surface membrane fluorescence* and the dominance of either kappa or lambda light chain expression on the cells was used as a criterion for the monoclonal B cell expansion in the disease

*Assay for T cells* The capacity of normal T cells to form spontaneous rosettes with sheep red blood cells (E RFC) (11) was used as a marker for T cells

*Statistical analysis* Data were analysed as means of triplicate cultures in terms of net cpm i e subtraction of the spontaneous cpm from the experimental cpm The S E M values of the triplicate cultures were within 10% of the means

Correlation analysis was performed according to Spearman s rank order correlation (12) which is non parametric In contrast to parametric tests such as *t* test in regression analysis this test is independent of whether or not the observations are normally distributed Spearman s correlation is recommended when the observations are continuous i e not characterized by a large number of ties at each rank In this investigation the observations (net cpm) were not normally distributed but continuous with no or few ties at the different ranks The correlation coefficients were regarded as significant if the probability values in a one sided test were less than 0 025

When comparing means of mitogen responses and hemoglobin (Hb) and thrombocyte (Tc) values of independent samples (2 groups of patients) logarithmic and absolute values were used in a two-sided *t* test (Student s *t* test) and probability values were regarded as significant when $p<0\ 05$ When estimating the degrees of freedom the difference of variance between the two groups was accounted for (22)

## RESULTS

### *Surface membrane Ig*

Cells from all patients except one could be classified with regard to the expression of surface membrane immunoglobulins All leukemic clones *expressed the heavy chains my or my/delta and* either of the light chains kappa or lambda Between 49–97% of the cells from the different patients were positive for either antikappa or antilambda sera whereas less than 1% of the cells expressed light chains of the nonleukemic light chain class

### *Clinical course*

During the observation period 10 patients required treatment due to development of severe anemia or thrombocytopenia and 1 because of sudden enlargement of lymph nodes with bronchial obstruction (group T) The latter patient died shortly after initiation of treatment Another patient died from septicemia after splenectomy which was performed in an attempt to correct thrombocytopenia (Fig 1) The mean Hb and Tc values in groups T and NT at the time of diagnosis (I) and after 6 months (II) (time of mitogen studies) are given in Fig 2 At the time of diagnosis no significant difference was noted between Hb or Tc values in the two groups However after 6 months Hb was significantly lower in group T and there was also a tendency towards lower Tc values signifying a more rapid progression of the disease

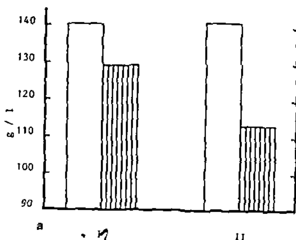
There were no significant differences in age sex or observation time between the two groups

### *Reproducibility of mitogenic patterns*

Short term (1 month) reproducibility of responses to the various mitogens was generally high However when studied 6 months after the first investigation the mean response to each mitogen except DxS 5 and 50 μg/ml was lower than initially This was significant for LPS 100 μg/ml anti $\beta_2$-m 1/10 and PPD 25 and 250 μg/ml (*t* test) However despite the subsequent depression of responses there was still a significant correlation ($0\ 001<p<0\ 025$) between the responses to each mitogenic concentration at the initial and second (after more than 6 months) investigation (Spearman rank correlation test) Thus the pattern of responses was generally unchanged but the magnitude was less at the second investigation The only exception to this finding was the response to DxS 50 μg/ml which varied independently at the first and second investigation

### *Correlation between mitogenic responses and hematological data*

Hb WBC Tc E RFC% and mitogenic responses were tested for possible correlations at the time of *diagnosis (I n=27) WBCs were inversely corre*lated to the E RFC% values ($p=0\ 003$) which was regarded as a dilution effect of the T cells due to the leukemic expansion Several individuals however demonstrated transient relative as well as absolute increases in the number of E RFC positive cells during the course of the disease thus demonstrating some expansion of the T cell population This has been described by several other investigators (4 13 21 23) In fact we did not observe a significant decrease in the E RFC values in spite of a significant increase in the WBCs during the present observation period PHA 1/1000 and 1/100 anti $\beta_2$-m 1/4 and PPD 2 5 25 and 250 μg/ml responses were positively correlated to the number of E RFC%



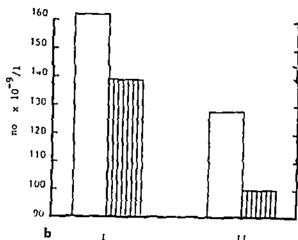




*Fig 2* (*a*) Mean Hb values in 10 CLL patients in group NT and in 9 patients in group T (2 patients were not re investigated at an interval of >6 months) *I*=investigation at the time of diagnosis *II*=investigation approximately 6 months later At investigation II the mean values for the two groups differed significantly ($p=0\ 006$) (*b*) Tc counts in patients in groups T and NT Mean values of the groups were not significantly different □ NT ▥=T

($p=0\ 020$) The influence of T cells on responses to these mitogens is in agreement with our previous data (20) However no correlations were seen be tween mitogenic responses WBC or E RFC% val ues on the one hand and Hb or Tc values on the other Neither the E RFC% values and WBC *nor differences in E RFC and WBC between the first and second investigation* correlated with progres sion of anemia or thrombocytopenia

*Correlations between mitogenic responses at the time of diagnosis (I) and clinical course (n=21)*

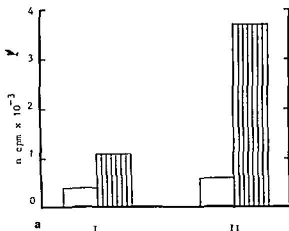
In group T ($n=11$) 10 patients required treatment because of progressing anemia and thrombo cytopenia and one patient because of severe lymph node enlargement leading to fatal bronchial obstruc tion The patients in group NT ($n=10$) followed for more than 10 months did not require treat ment during the observation period

Observation periods and intervals between diag nosis and initiation of treatment are given in Fig 1 The mean values of mitogenic responses in the two groups were compared in a two-sided $t$ test based on logarithmic values Geometrical mean values are presented in Fig 3 Mean values of DxS 5 μg/ml and LPS 2 5 25, 100 μg/ml responses were signifi cantly higher in group T than in group NT In a previous report responses in CLL cultures to LPS and DxS 5 μg/ml show covariance (20) LPS 100 μg/ml responses in cells from 9 of these 11 patients lay between 9 6 and 47 $5\times10^{-3}$ net cpm (mean 19000) at the time of diagnosis (I) Cells from only 2 patients in the group did not respond to LPS (Fig 1) In group NT ($n\approx10$) responses to LPS 100 μg/ml did not exceed 1 $4\times10^{-3}$ net cpm in 9 patients (Fig 1) The variance of responses to LPS and DxS was significantly greater ($p<0\ 001$) in group T which for statistical reasons decreased the significance levels when the mean values in groups T and NT were compared The mean WBC and E RFC% val ues did not differ between the two groups

These results were also confirmed in a $t$ test based on *absolute* values However in this test responses to *PHA 1/1000* were also significantly different between the two groups being *lower* in group T than in group NT ($p=0\ 05$) Furthermore anti $\beta_2$ m 1/40 responses were higher in group T than in group NT ($p=0\ 04$)

*Correlations between kappa and lambda surface membrane Ig light chain restriction and clinical course*

Of 9 patients with lambda CLL 7 needed treat ment against only 4 of 11 kappa patients (Fig 1) This difference though not significant in a $\chi^2$ test tends to support earlier findings (13) As expected mitogenic responses were not correlated to the kappa and lambda light chain restriction



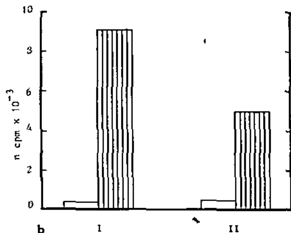




*Fig 3* (*a*) Geometric mean values of responses to DxS 5 μg/ml in cells from 10 patients in group NT and in 9 patients in group T (2 patients were not reinvestigated at an interval of >6 months) Responses analysed at the time of diagnosis (*I*) and at a second investigation (*II*) more than 6 months later At the first investigation the mean values were significantly different between the two groups ($p=0\ 049$) However the variance of responses was significantly greater in the group T ($p<0\ 001$) on both occasions (*b*) Geometric mean responses to LPS 25 μg/ml Responses in group T were significantly higher both at the time of diagnosis (*I*) ($p=0\ 006$) and at investigation II ($p=0\ 051$) At investigation II the variance in group T was significantly greater than in group NT on the $p=4\ 4\times10^{-4}$ level □=NT ▥ T



## DISCUSSION

Our results show firstly that stimulation of lymphocytes in CLL with a battery of mitogens gives rise to relatively specific and reproducible response patterns characteristic for each patient However the magnitude of the responses in most cases decreased with progression of the disease while the response pattern for each patient remained mainly unchanged Secondly the patterns appear to be of value for prediction of prognosis

The mitogenic response patterns are probably the result of complex mechanisms involving both functional properties of the leukemic cells and secondary interactions of other cells such as T cells as reported previously (20) We found evidence for a considerable influence of the presence of T cells on responses in CLL cultures to B cell activators In the present study responses to many mitogens were also positively correlated to the proportion of T cells and the inverse numbers of peripheral WBC at the time of blood sampling Thus the observed decrease in responses during the course of the disease can be explained by a successive decrease in the relative proportion of T cells due to the leukemic expansion The T cells present in CLL have recently been shown to lack allogeneic helper activity on the B cell production of Ig (3) It was suggested that the T cells might develop tolerance to activation because of chronic exposure to excessive amounts of autologous Ia antigens present on the CLL B cell clone Thus the development of functionally abnormal T cells might be another explanation for the observed decreases in mitogenic responses with time (22)

In many cases however the relative as well as the absolute number of T cells were *increased* for at least a period of the clinical course An absolute increase in the E RFC positive cell population has also been reported by several others (4 13 21 23) but the mechanism is unclear One possibility would be a compensatory requirement for more T cells for the T–B cell interaction in the normal immune response Another interesting possibility would be that the leukemic transformation event might occur in a common precursor of the T–B cell lineages (4) Assuming weaker mitogenic responses of the hypothetical CLL T lymphocytes this mechanism might also be consistent with the observed decrease in responses with time

Our results imply that it might be clinically relevant to determine in vitro responses of CLL lymphocytes to polyclonal B cell activating substances such as DxS and LPS Thus low responses to PHA on the one hand and high responses to DxS

low concentrations of anti $\beta_2$ m and in particular LPS on the other are associated with a more rapid development of clinical symptoms particularly thrombocytopenia and anemia We have recently presented a tentative model for the human B lymphocyte differentiation based on mitogen responsiveness of CLL cells (20) DxS and low concentrations of anti $\beta_2$-m are believed to stimulate immature cells, LPS intermediate cells and PPD PHA and high concentrations of anti $\beta$ m more mature B cells A similar sequence for optimal stimulation of maturing murine lymphocytes has previously been reported (7) In cell cultures from most patients however the DxS and LPS responses were low compared to the responses to PHA PPD and high concentrations of anti $\beta$ m even in cells from patients with relatively high DxS and LPS responses Thus CLL cells from each patient did not seem to represent just one maturation stage We argued that cells in different maturation stages were present within each leukemic clone and that cells in earlier differentiation stages were generally present in only small numbers Thus the high DxS and LPS responsive leukemias observed in this study should be characterized by a comparatively high proportion of relatively immature cells It is tempting to speculate that immaturity was the reason for poor prognosis This is supported by results in a recent investigation (5) which shows that patients with large CLL cells and a high proportion of CLL cells with nucleoli have a poor prognosis

It is not likely that the patients who required treatment in this study had had their disease considerably longer before diagnosis than those who did not require treatment We did not observe any LPS non responder who later became a responder On the contrary the mean LPS response decreased with time in group T Furthermore the WBC was not higher in group T or in LPS responders than in group NT or LPS non responders respectively Thus we do not believe that the early requirement for treatment in group T was due to late diagnosis On the contrary it appears that LPS responsiveness indicates a more aggressive disease with more immature cells in the blood even at an early stage

Our results support a previous work (13) which has shown that SmIg lambda chain expression is frequently associated with poor prognosis However there was no clear correlation between LPS responsiveness and lambda chain expression Cells from some patients responded to LPS and expressed kappa chains while some LPS non responder expressed lambda chains However all patients with both lambda chain expression and LPS responsiveness were in group T The determination of both these parameters should therefore be of value for the prognostic characterization of CLL cells

## ACKNOWLEDGEMENTS

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## REFERENCES

1 Boggs D R Sofferman S A Wintrobe M & Cartwright G E Factors influencing the duration of survival of patients with chronic lymphocytic leukemia Am J Med 40 243 1966
2 Boyum A Separation of leukocytes from blood and bone marrow Scand J Clin Lab Invest (Suppl) 1 21 1968
3 Chiorazzi N Fu S M Montazeri G Kunkel H G Rai K R & Gee T T Cell helper defect in patients with chronic lymphocytic leukemia J Immunol In press 1979
4 Davis S The variable pattern of circulating lymphocyte subpopulations in chronic lymphocytic leukemia N Engl J Med 21 1150 1976
5 Dubner H Crowley J & Schilling R Prognostic value of nucleoli and cell size in chronic lymphocytic leukemia Am J Hematol 4 337 1978
6 Green R A & Dixon H Expectancy for life in chronic lymphatic leukemia Blood 25 23 1965
7 Gronowicz E & Coutinho A Functional analysis of B cell heterogeneity Transplant Rev 24 3 1975
8 Hellstrom U Mellstedt H Perlmann P Holm G & Pettersson P Receptors for helix pomatia A hemagglutinin on leukemic lymphocytes from patients with CLL Clin Exp Immunol 26 196 1976
9 Huguley C M Survey of current therapy and of problems in chronic leukemia In Leukemia lymphoma p 317 Year Book Medical Publishers Chicago 1970
10 Idestrom K Engstedt L Gahrton G Holm G Johansson B Killander A Killander D Mellstedt H Robèrt K H & Wadman B Provning av Prednimustin (Leo 1031) vid kronisk lymfatisk leukemi och hogt differentierade lymfocytara lymfom Svenska Lakaresallskapets riksstamma 1976
11 Jondal M Holm G & Wigzell H Surface markers on human B and T lymphocytes forming non immune rosettes with sheep red blood cells A human T lymphocyte marker J Exp Med 136 207 1972
12 Kendahl M G Rank correlation methods 2nd ed Charles Griffin London 1955

13 Mellstedt H Pettersson D & Holm G Lymphocyte subpopulations in chronic lymphatic leukemia (CLL) Acta Med Scand 204 485 1978
14 Persson U Hammarström L Moller E Moller G & Smith C I E The role of adherent cells in B and T lymphocyte activation Immunol Rev 40 78 1978
15 Pettersson D Mellstedt H & Holm G IgG on human blood lymphocytes studied by immuno fluorescence Scand J Immunol In press 1978
16 Rai K Sawitsky A Cronkite E Chanana A Levy R N & Pasternack B Clinical staging of chronic lymphocytic leukemia Blood 46 219 1975
17 Robèrt K H PHA induced soluble factor(s) can activate B cells from patients with chronic lymphatic leukemia Clin Exp Immunol 37 517 1979
18 Robèrt K H Bird A G & Moller E Mitogen induced differentiation of human CLL lymphocytes to antibody secreting cells Scand J Immunol In press 1979
19 Robert K H Möller E Gahrton G Eriksson H & Nilsson B B cell activation of peripheral blood lymphocytes from patients with chronic lymphatic leukemia Clin Exp Immunol 33 302 1978
20 Robèrt K H & Nilsson B Covariances of mitogenic responses in leukemic blood lymphocytes a functional marker system for the human B lymphocyte differentiation Scand J Immunol 10 127 197
21 Salsano F Frøland S Natvig J & Mandelli F Chronic lymphocytic leukemia studies on the effect of drug treatment on different lymphocytic subpopulations Scand J Immunol 5 1185 1976
22 Satterthwaite F An approximate distribution of estimates of variance components Biometrics 2 11 1946
23 Traczyk Z Ciesluk S Litwin J & Arczynska E Evaluation of immunologic reactivity of lymphocytes from patients with chronic lymphocytic leukemia various clinical conditions Arch Immunol Ther Ex 23 589 1975

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# Diagnostic Significance of Lysosomal Enzymes in Different Types of Leukemias

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ABSTRACT The activities of seven different leukocyte hydrolases were stud ed in 19 patients and ten controls There was a strong positive correlat on between the monocyte count and the activities of the lysosomal enzymes (N acetyl β-glucosaminidase α fucosidase β-galactosidase and α mannosidase) High α fucosidase and α mannosidase activities were also found in the eosinophilic granulocytes Using simple commercially ava lable synthetic substrates it is possible to study the activities of the lysosomal enzymes in different types of leukemias and to re cognize the monocytic leukemias even when they present with very immature precursor cells in the peripheral blood

*Key wo ds* hydrolases leukocytes leukem a lysosomal enzymes morpholog cal classif cat on peripheral blood phosphatases

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During the last decade there has been increas ng emphas s on the necess ty of separat ng the acute leukemias into several cytolog cal subgroups Th s has led to different therapeutic reg mes somet mes with promising results Often the diagnosis of leukem a is based on purely morphological criteria but recently Tan and Lamberg (22) showed that there s a marked stain-dependent variation in the appearance of a blast cell population The authors caut oned aga nst general sat ons regard ng the morphology as the only criterion for the select on of therapy The application of cytochem cal methods to the study of leukemias has lead to the demonstra t on of characterist c patterns in the various types of cells (1 20) but there are still many cases where the exact diagnosis must be tentative

Stud es of certa n enzymes in the leukocytes e g various hydrolases have also been of d agnost c significance in some types of leukem as and the present study was undertaken to f nd out whether the activities of some hydrolases especially those w th a d st nct lysosomal d stribut on (17) could give further d agnost c informat on

## NOMENCLATURE

The cells were classif ed according to the criteria given by He lmeyer and Begemann (11) and Sjogren (21) The clas sif cat on of the leukem as is that of Galton and Dac e (9) and Sjogren (21)

## SUBJECTS AND METHODS

### *Controls*

Five men and f ve women (laboratory personnel) aged 21 57 years were used as controls Peripheral blood data are g ven in Table I

### *Pa ents*

Age sex peripheral blood data and diagnoses are shown in Table I All the leukem c pat ents were nvest gated at the t me of d agnosis

### *Morphological n est gat ons*

May-Grunwald-G emsa stained blood films from all pa t ents and concomitant bone marrow smears from 15 pa t ents were ava lable A differential count of 1000 nu cleated cells n each smear was performed by one of the authors (U S) without knowledge of the b ochem cal data

### *H d olase assay*

Of a leukocyte homogenate (5000-10000 leukocytes/μl) prepared as described earl er (12) 25 μl were ncubated w th 25 μl 1 M c trate buffer (pH values are g ven n Tables II and III) and 100 μl 1 mM 4-methylumbelliferyl glycos des (Koch L ght Lab Colnbrook UK) in d st lled water For assay of alkaline phosphatase 0 2 M glycine buffer pH 10 5 was used Assays were carried out at 37°C for various t mes up to 60 m n The reactions were stopped and read as described prev ously (17)

Table I *Hematologic data on 19 patients and 10 controls*
N = neutrophils E = eosinophils L = lymphocytes M = monocytes PMo = promonocytes MB = myeloblasts MC = promyelocytes metamyelocytes LB = lymphoblasts AML = acute myeloblastic leukemia AMMoL = acute myelomonocytic leukemia, CMMoL = chronic myelomonocytic leukemia, AMoL = acute monocytic leukemia ALL = acute lymphoblastic leukemia CLL = chronic lymphocytic leukemia the subtypes according to the FAB classification system (3 21) are also given

| Pat. no | Sex | Age (y) | WBC (×10⁹/l) | N (%) | E (%) | L (%) | M (%) | PMo (%) | MB (%) | MC (%) | LB (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ♀ | 29 | 5 8 | 61 0 | 1 2 | 29 0 | 7 0 | 1 6 | | | |
| 2 | ♂ | 54 | 5 8 | 42 8 | 1 0 | 39 0 | 16 2 | 1 0 | | | |
| 3 | ♂ | 66 | 38 5 | 29 2 | 61 8 | 5 5 | 3 0 | | | | |
| 4 | ♂ | 28 | 9 4 | 33 8 | 15 3 | 44 8 | 4.5 | 1 1 | | | |
| 5 | ♂ | 62 | 5 4 | 39 4 | 1 2 | 22 0 | 4 8 | | | 27 6 | 5 0 |
| 6 | ♀ | 74 | 29 5 | 6 7 | 0 2 | 28 8 | 0 1 | 4 8 | 57 9 | 1 5 | |
| 7 | ♀ | 63 | 227 0 | 12 6 | 0 4 | 34 0 | 1 4 | 9 4 | 36 1 | 5 9 | |
| 8 | ♂ | 38 | 26 2 | 7 6 | 0 1 | 5 6 | 0 2 | 4 4 | 79 4 | 2 7 | |
| 9 | ♀ | 27 | 64 0 | 9 7 | 3 1 | 30 3 | 24 3 | 15 9 | 16 0 | 0 7 | |
| 10 | ♀ | 54 | 24 3 | 10 5 | 0 2 | 14 6 | 0 2 | 7 5 | 57 2 | 9 8 | |
| 11 | ♂ | 74 | 14 1 | 35 7 | 1.5 | 28 1 | 29 1 | 4 9 | | 0 2 | |
| 12 | ♀ | 74 | 7.5 | 21 8 | | 19 8 | 22 6 | 34 0 | 1 6 | 0 2 | |
| 13 | ♂ | 48 | 8 4 | 3 7 | 1 8 | 33 2 | 2 5 | 22 1 | 31 4 | 5 3 | |
| 14 | ♂ | 77 | 152 0 | 1 2 | 0 1 | 4 7 | 0 4 | 53 8 | 37 6 | 2 2 | |
| 15 | ♂ | 43 | 1 9 | 3 8 | 0 5 | 64 5 | 3 2 | 0 8 | | 0 5 | 26 5 |
| 16 | ♀ | 26 | 18.5 | 2 6 | | 33 7 | 0 3 | 0 5 | | 1 1 | 61 8 |
| 17 | ♂ | 57 | 43 3 | 4 4 | 0 2 | 94 6 | 0 8 | | | | |
| 18 | ♀ | 82 | 23 4 | 18.2 | 4 4 | 74 6 | 2 8 | | | | |
| 19 | ♀ | 71 | 133 0 | 3 1 | 0 1 | 95 8 | 1 0 | | | | |
| Controls (range) | | | 5 6–9 9 | 48 2–68 3 | 0.5–7 7 | 21 4–45 8 | 3 4–8 3 | | | | |

Table II *Enzymic activities of 19 patients and 10 controls (U/10⁹ WBC)*

| | | | N acetyl-β-glucosaminidase | | | | |
|---|---|---|---|---|---|---|---|
| Pat. no | Acid phosphatase | Alkaline phosphatase | Paranitrophenyl substrate | Methylumbelliferyl substrate | α Fucosidase | | β-Galactosidase |
| 1 | 1 97 | 1 18 | 5 90 | 1 66 | 0 017 | 0 080 | 0 090 |
| 2 | 1 47 | 0 25 | 4 15 | 2 10 | 0 051 | 0 11 | 0 13 |
| 3 | 1 08 | 0 12 | 1 64 | 0 50 | 0 095 | 0 16 | 0 13 |
| 4 | 0 62 | 0 47 | 6 75 | 1 07 | 0 022 | 0 14 | 0 030 |
| 5 | 1 49 | 1 55 | 2 09 | 1 10 | 0 009 | 0 056 | 0 18 |
| 6 | 0 46 | 0 23 | 1 73 | 0 99 | 0 011 | 0 074 | 0 080 |
| 7 | 0 17 | 0 030 | 3 30 | 0 93 | 0 016 | 0 090 | 0 12 |
| 8 | 0 46 | 0 027 | 2 79 | 0 99 | 0 023 | 0 16 | 0 37 |
| 9 | 1 04 | 0 020 | 4 06 | 1 37 | 0 040 | 0 14 | 0 22 |
| 10 | 1 12 | 0 030 | 3 75 | 1.57 | 0 019 | 0 17 | 0 36 |
| 11 | 0 93 | 0 10 | 3 90 | 1 43 | 0 032 | 0 090 | 0 21 |
| 12 | 2.26 | 0 49 | 8.54 | 3.26 | 0 067 | 0 20 | 0 43 |
| 13 | 0 63 | 0 036 | 3 80 | 1 17 | 0 060 | 0 21 | 0 047 |
| 14 | 1 14 | 0 032 | 4 72 | 2 26 | 0 054 | 0 14 | 0 49 |
| 15 | 1 68 | 0 050 | – | 0 16 | 0 005 | 0 060 | 0 030 |
| 16 | 0.23 | 0 035 | 0 73 | 0 14 | 0 011 | 0 042 | 0 021 |
| 17 | 0 090 | 0 060 | 1 85 | 0 15 | 0 004 | 0 030 | 0 030 |
| 18 | 0.55 | 0 18 | 3 10 | 0 71 | 0 022 | 0 12 | 0 12 |
| 19 | 0.20 | 0 040 | 1 64 | 0 32 | 0 017 | 0 025 | 0 058 |
| Controls (range) | 0.34–1 75 | 0 0–0 60 | 1 20–3 90 | 0 35–1 12 | 0 010–0 033 | 0 073–0 13 | 0 080–0 14 |
| pH | 5 0 | 10.5 | 4.5 | 4.5 | 4 0 | 5 5 | 4 5 |

Table I *Hematologic data on 19 patients and 10 controls*
N = neutrophils, E = eosinophils, L = lymphocytes M = monocytes PMo = promonocytes MB = myeloblasts MC = promyelocytes-metamyelocytes LB = lymphoblasts AML = acute myeloblastic leukemia, AMMoL = acute myelomonocytic leukemia, CMMoL = chronic myelomonocytic leukemia, AMoL = acute monocytic leukemia, ALL = acute lymphoblastic leukemia, CLL = chronic lymphocytic leukemia, the subtypes according to the FAB classification system (3 21) are also given

| Pat. no | Sex | Age (y) | WBC (×10⁹/l) | N (%) | E (%) | L (%) | M (%) | PMo (%) | MB (%) | MC (%) | LB (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ♀ | 29 | 5.8 | 61.0 | 1.2 | 29.0 | 7.0 | 1.6 | | | |
| 2 | ♂ | 54 | 5.8 | 42.8 | 1.0 | 39.0 | 16.2 | 1.0 | | | |
| 3 | ♂ | 66 | 38.5 | 29.2 | 61.8 | 5.5 | 3.0 | | | | |
| 4 | ♂ | 28 | 9.4 | 33.8 | 15.3 | 44.8 | 4.5 | 1.1 | | | |
| 5 | ♂ | 62 | 5.4 | 39.4 | 1.2 | 22.0 | 4.8 | | | 27.6 | 5.0 |
| 6 | ♀ | 74 | 29.5 | 6.7 | 0.2 | 28.8 | 0.1 | 4.8 | 57.9 | 1.5 | |
| 7 | ♀ | 63 | 227.0 | 12.6 | 0.4 | 34.0 | 1.4 | 9.4 | 36.1 | 5.9 | |
| 8 | ♂ | 38 | 26.2 | 7.6 | 0.1 | 5.6 | 0.2 | 4.4 | 79.4 | 2.7 | |
| 9 | ♀ | 27 | 64.0 | 9.7 | 3.1 | 30.3 | 24.3 | 15.9 | 16.0 | 0.7 | |
| 10 | ♀ | 54 | 24.3 | 10.5 | 0.2 | 14.6 | 0.2 | 7.5 | 57.2 | 9.8 | |
| 11 | ♂ | 74 | 14.1 | 35.7 | 1.5 | 28.1 | 29.1 | 4.9 | | 0.2 | |
| 12 | ♀ | 74 | 7.5 | 21.8 | | 19.8 | 22.6 | 34.0 | 1.6 | 0.2 | |
| 13 | ♂ | 48 | 8.4 | 3.7 | 1.8 | 33.2 | 2.5 | 22.1 | 31.4 | 5.3 | |
| 14 | ♂ | 77 | 152.0 | 1.2 | 0.1 | 4.7 | 0.4 | 53.8 | 37.6 | 2.2 | |
| 15 | ♂ | 43 | 1.9 | 3.8 | 0.5 | 64.5 | 3.2 | 0.8 | | 0.5 | 26.5 |
| 16 | ♀ | 26 | 18.5 | 2.6 | | 33.7 | 0.3 | 0.5 | | 1.1 | 61.8 |
| 17 | ♂ | 57 | 43.3 | 4.4 | 0.2 | 94.6 | 0.8 | | | | |
| 18 | ♀ | 82 | 23.4 | 18.2 | 4.4 | 74.6 | 2.8 | | | | |
| 19 | ♀ | 71 | 133.0 | 3.1 | 0.1 | 95.8 | 1.0 | | | | |
| Controls (range) | | | 5.6–9.9 | 48.2–68.3 | 0.5–7.7 | 21.4–45.8 | 3.4–8.3 | | | | |

Table II *Enzymic activities of 19 patients and 10 controls (U/10⁹ WBC)*

| Pat. no | Acid phosphatase | Alkaline phosphatase | N acetyl β-glucosaminidase: Paranitrophenyl substrate | N acetyl β-glucosaminidase: Methylumbelliferyl substrate | α Fucosidase | | β-Galactosidase |
|---|---|---|---|---|---|---|---|
| 1 | 1.97 | 1.18 | 5.90 | 1.66 | 0.017 | 0.060 | 0.090 |
| 2 | 1.47 | 0.25 | 4.15 | 2.10 | 0.051 | 0.11 | 0.13 |
| 3 | 1.08 | 0.12 | 1.64 | 0.50 | 0.095 | 0.16 | 0.13 |
| 4 | 0.62 | 0.47 | 6.75 | 1.07 | 0.022 | 0.14 | 0.090 |
| 5 | 1.49 | 1.55 | 2.09 | 1.10 | 0.009 | 0.056 | 0.18 |
| 6 | 0.46 | 0.23 | 1.73 | 0.99 | 0.011 | 0.074 | 0.060 |
| 7 | 0.17 | 0.050 | 3.30 | 0.93 | 0.016 | 0.090 | 0.12 |
| 8 | 0.46 | 0.027 | 2.79 | 0.99 | 0.023 | 0.16 | 0.37 |
| 9 | 1.04 | 0.020 | 4.06 | 1.37 | 0.040 | 0.14 | 0.22 |
| 10 | 1.12 | 0.030 | 3.75 | 1.57 | 0.019 | 0.17 | 0.36 |
| 11 | 0.93 | 0.10 | 3.90 | 1.43 | 0.032 | 0.090 | 0.21 |
| 12 | 2.26 | 0.49 | 8.54 | 3.26 | 0.067 | 0.20 | 0.43 |
| 13 | 0.63 | 0.056 | 3.60 | 1.17 | 0.060 | 0.21 | 0.047 |
| 14 | 1.14 | 0.032 | 4.72 | 2.26 | 0.054 | 0.14 | 0.49 |
| 15 | 1.68 | 0.050 | – | 0.16 | 0.005 | 0.060 | 0.050 |
| 16 | 0.23 | 0.035 | 0.73 | 0.14 | 0.011 | 0.042 | 0.021 |
| 17 | 0.090 | 0.060 | 1.85 | 0.15 | 0.004 | 0.050 | 0.050 |
| 18 | 0.55 | 0.18 | 3.10 | 0.71 | 0.022 | 0.12 | 0.12 |
| 19 | 0.20 | 0.040 | 1.64 | 0.32 | 0.017 | 0.025 | 0.058 |
| Controls (range) | 0.34–1.75 | 0.040–0.60 | 1.20–3.90 | 0.35–1.12 | 0.010–0.033 | 0.073–0.13 | 0.080–0.14 |
| pH | 5.0 | 10.5 | 4.5 | 4.5 | 4.0 | 5.5 | 4.5 |

Table I *Hematolog*
N neutroph ls E
promyelocytes metam
nocyt c leukemia CM
lymphoblastic leukem a
21) are also g ven

| Pat no | Sex | Age (y ) |
|---|---|---|
| 1 | ♀ | [illegible]9 |
| 2 | ♂ | 54 |
| 3 | ♂ | 66 |
| 4 | ♂ | 28 |
| 5 | ♂ | 6[illegible] |
| 6 | ♀ | 74 |
| 7 | ♀ | 63 |
| 8 | ♂ | 38 |
| 9 | ♀ | 27 |
| 10 | ♀ | 54 |
| 11 | ♂ | 74 |
| 12 | ♀ | 74 |
| 13 | ♂ | 48 |
| 14 | ♂ | 77 |
| 15 | ♂ | 43 |
| 16 | ♀ | 26 |
| 17 | ♂ | 57 |
| 18 | ♀ | 8[illegible] |
| 19 | ♀ | 71 |
| Controls (range) | | |

Table II *En ymic act*

| Pat no | Acid phosphatase | [illegible] |
|---|---|---|
| 1 | 1 97 | 1 |
| 2 | 1 47 | [illegible] |
| 3 | 1 08 | 0 |
| 4 | 0 6[illegible] | 0 |
| 5 | 1 49 | 1 |
| 6 | 0 46 | 0 [illegible] |
| 7 | 0 17 | 0 0 |
| 8 | 0 46 | 0 0 |
| 9 | 1 04 | 0 0[illegible] |
| 10 | 1 12 | 0 03 |
| 11 | 0 93 | 0 10 |
| 12 | [illegible] 26 | 0 49 |
| 13 | 0 63 | 0 036 |
| 14 | 1 14 | 0 032 |
| 15 | 1 68 | 0 050 |
| 16 | 0 23 | 0 035 |
| 17 | 0 090 | 0 060 |
| 18 | 0 55 | 0 18 |
| 19 | 0 20 | 0 040 |
| Controls (range) | 0 34–1 75 | 0 040–0 60 |
| pH | 5 0 | 10 5 |

[illegible] has been found to
[illegible] B l mph cytes when
[illegible] (16) and the im
[illegible] leukemia seem
[illegible] than the mature cells of
[illegible] (7) Rather h g
[illegible] in cases with a reactive
[illegible] a decreased activity
[illegible] leukemia (8 14) A
[illegible] has been reported in cases
[illegible] various inflammatory d s
[illegible] activit es in monocyt c
[illegible] activity s th
[illegible] rent d so
[illegible] of
[illegible] be
[illegible] of d
[illegible] f t
[illegible]

Table III *Relations between enzymic activities (U/10⁹ WBC) and percentages of different types of WBC*
Figures give the Spearman rank correlation coefficients and the degrees of significance

| | | Cell types* | | | | | |
|---|---|---|---|---|---|---|---|
| Enzymes | pH | N | N+MC | M+PMo | E+M<br>+PMo | N+MC<br>+E+M<br>+PMo | L+LB |
| Acid phosphatase | 5 0 | 0 242 | 0 302 | 0 391<br><0 05 | 0 345 | 0 428<br><0 05 | −0 238 |
| Alkaline phosphatase | 10 5 | 0 666<br><0 001 | 0 667<br><0 001 | −0 030 | 0 082 | 0 620<br><0 01 | 0 091 |
| N Acetyl β-glucosaminidase | | | | | | | |
| Paranitrophenyl substrate | 4 5 | 0 036 | 0 068 | 0 815<br><0 001 | 0 656<br><0 01 | 0 194 | −0 247 |
| Methylumbelliferyl substrate | 4 5 | 0 096 | 0 175 | 0 868<br><0 001 | 0 645<br><0 01 | 0 334 | −0 534<br><0 01 |
| α Fucosidase | 4 0 | −0 198 | −0 185 | 0 537<br><0 01 | 0 748<br><0 001 | 0 204 | −0 423<br><0 05 |
| | 5.5 | 0 016 | 0 028 | 0 518<br><0 01 | 0 665<br><0 001 | 0 290 | −0 458<br><0 05 |
| β-Galactosidase | 4.5 | 0 036 | 0 098 | 0 553<br><0 01 | 0 546<br><0 01 | 0 357 | −0 725<br><0 001 |
| β-Glucuronidase | 4 5 | 0 262 | 0 234 | 0 326 | 0 325 | 0 347 | −0 500<br><0 02 |
| α Mannosidase | 4 5 | 0 141 | 0 202 | 0 517<br><0 01 | 0 685<br><0 001 | 0 438<br><0 05 | −0 627<br><0 01 |
| | 5.5 | 0 126 | 0 166 | 0 382 | 0 617<br><0 01 | 0 395<br><0 05 | −0 579<br><0 01 |

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As the acid hydrolases are components of the lysosomal system they are suited to express differences between phagocytic and non phagocytic cells (7) Thus raised activities of N acetyl β

Table III *Relations between enzymic activities (U/10⁹ WBC) and percentages of different types of WBC*
Figures give the Spearman rank correlation coefficients and the degrees of significance

| Enzymes | pH | Cell types[a] N | N+MC | M+PMo | E+M +PMo | N+MC +E+M +PMo | L+LB |
|---|---|---|---|---|---|---|---|
| Acid phosphatase | 5 0 | 0 242 | 0 302 | 0 391<br><0 05 | 0 345 | 0 428<br><0 05 | −0 238 |
| Alkaline phosphatase | 10 5 | 0 666<br><0 001 | 0 667<br><0 001 | −0 030 | 0 082 | 0 620<br><0 01 | 0 091 |
| N Acetyl β glucosaminidase | | | | | | | |
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| | 5 5 | 0 126 | 0 186 | 0 382 | 0 617 | 0 395 | −0 579 |
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18 Rosales C L Bennett J M & Rutenburg A M Histochemical demon trat on of leucocyte ac d phosphatase in heal h and in d sease Br J Haematol 12 17? 1966
19 Ross ter R J & Wong E β glucuron dase of human wh te blood cells Blood 5 864 1950
20 Schmalzl F & Braunste ner H The appl cation of cytochem d to the study of acute leukem a A rev ew t na 145 209 1971
21 Sjog en L y n m el nd leukaem a A study of ? nd J Haematol 0 159 19 5
22 Tan H K A e J D D gnos s of ac e leukem a V f m rpho g criteria Am J Cl n Pa h 168 4 1977

18 Rosales C L, Bennett J M & Rutenburg A M Histochemical demonstration of leucocyte acid phosphatase in health and in disease Br J Haematol 12: 172 1966
19 Rossiter R J & Wong E β-glucuronidase of human white blood cells Blood 5 864 1950
20 Schmalzl F & Braunsteiner H The application of cytochemi[illegible] to the study of acute leukemia A review [illegible] 45 209 1971
21 Sjogren U [illegible] in myeloid leukaemias A study of 2[illegible] cases [illegible] and J Haematol 20 159 197[illegible]
22 Tan H K [illegible] J D Diagnosis of acute leukemia Variability of morphologic criteria Am J Clin Pathol 6[illegible] 1977

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# Scalene Node Biopsy in Sarcoidosis

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ABSTRACT Scalene node biopsy was performed by a trained surgeon in 39 patients with established sarcoidosis The diagnostic yield in this group was compared with the results in 43 patients with established sarcoidosis who had been subjected to routine scalene node biopsies at the same clinic Sarcoid tissue was found in 82% of the patients operated on by the trained surgeon compared with 47% in the other group It is concluded that, in the hand of a trained surgeon with a good operating technique, scalene node biopsy is a good alternative for obtaining tissue from sarcoidosis patients for histopathological examination

*Key words* sarcoidosis scalene node biopsy
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According to the Second International Conference on Sarcoidosis (1960) the diagnosis of sarcoidosis is established for clinical purposes in patients who have consistent clinical features together with biopsy evidence of epithelioid tubercles or a positive Kveim test Several methods have been tried for obtaining biopsy material for the diagnosis of sarcoidosis If palpable lymph nodes skin lesions or enlarged parotid glands are present biopsy from such abnormalities gives a high percentage of sarcoid tissue (6) If no such abnormalities are present scalene node biopsy as described by Daniels (5) has proved useful in obtaining biopsy material In patients with sarcoidosis the frequency of positive biopsies obtained by this method varies in the literature from 32 to 83% (7 8)

The purpose of this study was to demonstrate the optimal yield of scalene node biopsy in sarcoidosis and to compare the results of the operation when performed by one interested surgeon or routinely

## PATIENTS AND METHODS

Scalene node biopsy was performed in 39 sarcoid patients (group A) diagnosed in 1977–78 by one surgeon with special interest in the operation There were 20 men and 19 women aged 22–78 years (mean 46) Seventeen of these patients had sarcoidosis stage I (hilar lymph adenopathy) 18 stage II (pulmonary infiltration with or without hilar lymphadenopathy) and four stage III (pulmonary fibrosis)

As a reference group we used 43 patients (group B) with sarcoidosis diagnosed in 1974–76 at our clinic They had undergone right-sided scalene node biopsy—performed by several surgeons—with routine extirpation of the fat pad There were 21 men and 22 women aged 25–76 years (mean 48) Thirteen patients had sarcoidosis stage I 23 stage II and seven stage III

All patients in both groups had a clinical picture and chest X ray typical of sarcoidosis Biopsy material from one or several organs showed non-caseating epithelioid cell granulomas without necrosis consistent with sarcoidosis The possibility of tuberculosis was excluded by sputum cultures and in group A also by culture of the lymph nodes from the scalene node biopsy specimen

A right sided scalene node biopsy was performed in all patients of group A by one and the same surgeon The patients were premedicated with 0 5–1 0 ml morphine scopolamine (10 mg/ml+0 4 mg/ml) subcutaneously The patient was placed in the supine position with the head turned to the left Infiltration anesthesia was induced by 20–40 ml of 0.5% Citanest Exedrine® Skin incision was made 2 cm above and parallel to the clavicle the platysma was incised transversally The anterior layer of the deep fascia of the neck was opened and the internal jugular vein and the omohyoid muscle were identified The triangular area between these structures is covered by the middle layer of the deep fascia of the neck which was opened and the fat pad lying on the scalenus anticus muscle was exposed The fat pad was dissected and several lymph nodes were removed during this dissection During the operation the internal jugular vein and the phrenic nerve were identified and protected against traumatic lesions A small rubber drain was placed in the wound which was closed stepwise

## RESULTS

The yield of positive biopsies in group A (patients diagnosed in 1977–78) was 82% and in group B (patients diagnosed in 1974–76) 47% The findings at scalene node biopsy in groups A and B are shown in Table I The distribution of positive biopsies in

Table I *Findings at scalene node biopsy*

| | Sarcoid lymph nodes | Non sarcoid lymph nodes Macroscopical | Microscopical | No lymph nodes | Positive biopsies (%) |
|---|---|---|---|---|---|
| Group A ($n$=39) | 32 | 6 | 1 | 0 | 82 |
| Group B ($n$=43) | 20 | 8 | 3 | 12 | 47 |

the two groups with regard to the stage of the disease is shown in Table II In group A scalene node biopsy failed to provide sarcoid tissue in seven patients Four of these had stage I sarcoidosis—two with an acute illness (Lofgren s syndrome)—and three stage II sarcoidosis Lymph node tissue was found in all patients in group A In group B sarcoid tissue was absent in 23 (53%) patients evenly distributed in the three sarcoid stages In 12 of the patients no lymph node tissue was obtained (Table II)

No complications were seen at scalene node biopsy in group A In group B one patient had a hypesthesia of the ventral part of the right thorax and two patients had small local hemorrhages which resolved spontaneously

## DISCUSSION

In the present study scalene node biopsy was positive in 82% of the patients operated on by one trained surgeon (group A) This is in accordance with the best results reported in the literature Thus Israel and Sones (6) found positive biopsies in 74% Bacharach (1) in 81% and Scadding (11) in 83% Such a good result however calls for a good operating technique This is postulated by Selroos (12) who in his series found only 55% positive biopsies and considered that the inferior results might be due to the admittedly difficult operation technique His results are similar to those in our group B (patients with routine scalene node biopsy) where sarcoid tissue was found in 47% of the patients

The difference in diagnostic yield between our two groups could be explained to a large extent by the simple fact that the former operations were carried out by only one surgeon with a particular interest in the operation However several other factors may have contributed to the difference The patient s position during surgery and good knowledge of the anatomical situation in the scalene area are of great importance (7) It is generally accepted (10) that the lymph from the right lung and the left lower lobe and lower mediastinum goes to the right scalene nodes and for this reason the scalene node biopsy in sarcoidosis should be performed on the right side The scalene fat pad does not always contain lymph nodes (2) and it is important not only to remove the fat pad itself but also to look for lymph nodes after the removal The biopsy procedure should if possible not be concluded until lymph node material has been found One great difference between our groups was the high frequency (26%) of patients in group B in whom no lymph nodes were found at scalene node biopsy The reason for this is probably that the operations were performed too shallowly or that the fat pad was just removed without the surgeon looking thoroughly for lymph nodes

In our series the diagnostic yield did not differ greatly with the stage of the disease In group A there was a tendency towards better results in stages II and III but even in stage I 77% of the patients had positive biopsies The result except for the lower diagnostic yield was the same in group B When looking at younger men mostly with stage I sarcoidosis Munkgaard and Neukirch (9) found positive Daniels biopsies in 20 of 34 patients (59%) and positive mediastinoscopies in 41 of 44 (93%) They postulated that mediastinoscopy is preferable in patients of this kind In our series however scalene node biopsy seems a good alternative even in stage I sarcoidosis

There were no complications in group A and only a few minor complications in group B after scalene node biopsy in the present study Serious complications have however been reported (3 13) Skinner (13) reported 6% of serious or fatal complications after scalene node biopsy in 186 patients The complications in his series occurred however in the

Table II *Number of positive biopsies/biopsies performed in different stages of sarcoidosis*

| | Stage of sarcoidosis I | II | III | Total |
|---|---|---|---|---|
| Group A | 13/17 | 15/18 | 4/4 | 32/39 |
| Group B | 6/13 | 10/23 | 4/7 | 20/43 |

group of malignant diseases and not in the sarcoid group

Scalene node biopsy is an important method for obtaining biopsy material in sarcoidosis The diagnostic yield in the present study was 82% positive biopsies This is a good result but somewhat lower than yielded by mediastinoscopy (4 9) and lung biopsy (6) In our opinion scalene node biopsy when performed correctly still has a place in the diagnostic procedure of sarcoidosis

## REFERENCES


1 Bacharach T Sarcoidosis A clinical review of 111 cases Am Rev Respir Dis 84 12 1961
2 Bennet V A & Carr D T Scalene lymph adenopathy a postmortal study Am Rev Tuberc 76 503 1957
3 Burger R L Boyd T F & Strieder J W Complications of scalene lymph node biopsy J Thorac Cardiovasc Surg 45 307 1963
4 Carlens E Mediastinoscopy Ann Otol Rhinol Laryngol 74 1102 1965
5 Daniels A C A method of biopsy useful in diagnosing certain intrathoracic diseases Dis Chest 16 360 1949
6 Israel Å L & Sones M Selection of biopsy procedures for sarcoidosis diagnosis Arch Intern Med 113 255 1964
7 Lillington G A & Jamplis R W Scalene node biopsy Ann Intern Med 59 101 1963
8 Lovgren S & Snellman B Principles and procedures for obtaining biopsies in sarcoidosis Acta Med Scand (Suppl) 425 225 1964
9 Munkgaard S & Neukirch F Comparison of biopsy procedures in intrathoracic sarcoidosis Acta Med Scand 205 179 1979
10 Rochlin D B & Enterline H Y Prescalene lymph node biopsies A report of 142 cases Am J Surg 96 372 1958
11 Scadding J G Sarcoidosis Eyre and Spottiswoode London 1967
12 Selroos O The frequency clinical picture and prognosis of pulmonary sarcoidosis in Finland Acta Med Scand (Suppl) 503 1969
13 Skinner D B Scalene lymph node biopsy Reappraisal of risks and indications N Engl J Med 268 1324 1963

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# 30 Min ACTH Stimulation Test as Predictor of Hypothalamic-Pituitary-Adrenocortical Function

*Comparison with Metyrapone Test*

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**ABSTRACT** In 32 subjects the hypothalamic pituitary adrenocortical (HPA) response to metyrapone was found to correlate significantly with the adrenocortical response to exogenous ACTH This report provides additional evidence suggesting that a 30 min exogenous ACTH stimulation test accurately predicts the integrated responsiveness of the HPA system to various stimuli

*Key words* ACTH stimulation test metyrapone test hypothalamic pituitary adrenocortical function adrenocortical function cortisol 11-deoxycortisol

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Although the ACTH stimulation test theoretically assesses only the adrenocortical function our recent results strongly suggest that the 30 min exogenous ACTH stimulation test accurately predicts the integrated function of the hypothalamic pituitary adrenocortical (HPA) system following various stimuli (6 7 9) In the present study this hypothesis has been tested further as we have compared the HPA response to metyrapone with the adrenocortical response to exogenous ACTH

## PATIENTS AND METHODS

In 32 patients a metyrapone test was followed after 24 h by a 30 min exogenous ACTH stimulation test The patients 20 males and 12 females evenly distributed within the age interval 18–84 years were admitted for elective surgery Hepatic and renal function was normal and none presented with signs of endocrine disorders Informed consent was obtained

Metyrapone ditartrate (Metopirone®) was given intravenously at a dose rate of 17 5 mg/kg b wt /h and at a volume rate of 100 ml/h 5% glucose in water between 8 a m and noon Blood samples for cortisol and 11 deoxycortisol (comp S) analysis were drawn from a cubital vein at 1–2 h intervals for 12 h The cortisol synthesis was completely blocked as proved by a fall in plasma cortisol levels to zero at noon The adrenocortical response to metyrapone was measured as the rise in the sum of plasma cortisol plus comp S concentrations according to principles described earlier (1)

The short ACTH test was started at 8 a m by giving 250 µg corticotrophin [1 24] (Synacthen®) intravenously Blood samples for cortisol determination were drawn at 0 and 30 min

In samples taken during the metyrapone test cortisol and comp S were determined by double isotope derivative technique (3) In samples taken during the short ACTH test cortisol (11 OHCS) was measured fluorimetrically (10)

For statistical analysis Spearman s rank correlation test was used

## RESULTS

Peak sum values of cortisol plus comp S were reached between 2 and 4 hours after termination of metyrapone infusion A significant correlation was found between peak sum of plasma cortisol plus comp S during metyrapone and the 30 min plasma cortisol value after exogenous ACTH injection ($R=0.61$ $p<0.001$) (Fig 1)

## DISCUSSION

In the present study negative feedback control served to trigger HPA stimulation by blocking cortisol synthesis with metyrapone A highly significant correlation was found between the 30 min cortisol value after exogenous ACTH and the result of the feedback controlled stimulation of the HPA

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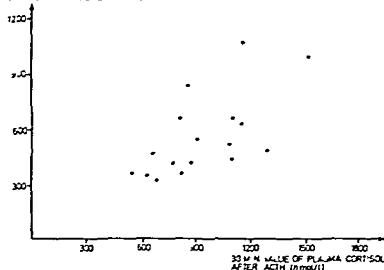



*Fig 1* Correlation between the peak sum of plasma cortisol plus comp S following metyrapone and the 30 min cortisol value after injection of 250 μg of ACTH

system In our recent studies the 30 min plasma cortisol level after injection of exogenous ACTH was also closely correlated to the peak plasma cortisol value during insulin-induced hypoglycaemia in normal subjects as well as in patients with pituitary disorders (7 9) Moreover a high correlation has been demonstrated between the HPA response to surgery and the adrenocortical response to 30 min exogenous ACTH testing in corticosteroid treated patients (6) These results suggest that the 30 min exogenous ACTH stimulation test accurately predicts the integrated HPA response to various stimuli Using a 2 hour ACTH stimulation test others have reached the same conclusion (8)

It has been observed that patients with hypothalamic pituitary impairment may have a normal *adrenocortical response to exogenous ACTH* but respond subnormally to hypoglycaemia and/or to metyrapone (4 5 8 11) In our recent studies on the relationship between the outcome of a hypoglycaemia test and a 30 min exogenous ACTH stimulation test (7 9) we did not find such a discrepancy The lack of correlation found by others may well be explained by a more prolonged ACTH stimulation compared with the 30 min exogenous ACTH stimulation test. Furthermore a complete block of cortisol synthesis by metyrapone was not assured (4 5 8 11)

All the plots but one in Fig. 1 are slightly to the right of the identity line This may be explained partly by the use of different methods for cortisol measurement during metyrapone and exogenous ACTH and partly by an accelerating effect of metyrapone on cortisol metabolism The fluorimetric analysis of cortisol gives slightly higher values than the double isotope derivative technique (1) Furthermore when metyrapone is administered the metabolic clearance rate of cortisol is about doubled which results in lower cortisol concentrations than expected (2)

## REFERENCES


1 Blichert Toft M Secretion of corticotrophin and somatotrophin by the senescent adenohypophysis in man Acta Endocrinol (Kbh) (Suppl) 195 51 53 74 1975

2 Blichert Toft M & Kehlet H Quantitation of the accelerating effect of metyrapone on cortisol metabolism. Clin Endocrinol (Oxf) 5 295 1976

3 Bojesen E Determination of 17 hydroxycorticosterone in peripheral plasma from dogs and humans with radioactive p iodophenylsulfonylic acid anhydride (pipsan) Scand J Clin Lab Invest 8 55 1965

4 Faglia, G Ambrosi B Beck Peccoz, P & Travaglini P Hypothalamic pituitary adrenal function in patients with pituitary tumours Acta Endocrinol (Kbh) 73 223 1973

5 Jenkins J S & Else W Pituitary adrenal function tests in patients with untreated pituitary tumours Lancet 2 940 1968

6 Kehlet, H & Binder C Value of an ACTH test in assessing hypothalamic pituitary adrenocortical function in glucocorticoid-treated patients Br Med J 2 147 1973

7 Kehlet H Blichert Toft M Lindholm J & Rasmussen P Short ACTH test in assessing hypothalamic pituitary adrenocortical function Br Med J 1 249 1976

8 Leisti S & Perheentupa J Two-hour adrenocorticotropic hormone test Accuracy in the evaluation of the hypothalamic pituitary adrenocortical axis Pediatr Res 12 272 1978
9 Lindholm J Kehlet H Blichert Toft M Dinesen B & Riishede J Reliability of the 30-minute ACTH test in assessing hypothalamic pituitary adrenal function J Clin Endocrinol Metab 47 272 1978
10 Nielsen E & Asfeldt V H Studies on the specificity of fluorimetric determination of plasma corticosteroids ad modum De Moor and Steeno Scand J Clin Lab Invest 20 185 1967
11 Nieman E A Landon J & Wynn V Endocrine function in patients with untreated chromophobe adenomas Q J Med 36 357 1967

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# Effect of an $H_2$-Receptor Blocking Agent on Diarrhoeas after Extensive Small Bowel Resection in Crohn's Disease

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**ABSTRACT** The effect of an $H_2$-receptor blocking agent cimetidine, on faecal losses of fluid electrolytes and fat was examined in 10 patients with Crohn s disease, who had diarrhoeas after extensive small bowel resection A randomized, double-blind and cross-over design was applied, and patients were hospitalized and on a defined diet during the study Cimetidine, 4×400 mg, significantly reduced diarrhoeal volumes by an average of 22% ($p<0.05$) and faecal sodium by 27% ($p<0.05$) Patients with severe diarrhoeas responded better to treatment No side-effects were recorded The reported data suggest that cimetidine may be useful in symptomatic treatment of patients with severe diarrhoeas after extensive ileal resection Due to deficient drug absorption, higher doses may be needed for optimal effect

*Key words* Crohn s disease bowel resection diarrhoea gastric acid secretion

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The further clinical course after extensive ileal resection in Crohn s disease may be characterized by voluminous diarrhoeas occurring either continuously with episodic aggravations or in sporadic attacks Although this sequel is seen in only a limited number of patients the severity of the symptoms and need for repeated hospitalizations for dehydration and electrolyte disturbances make it a quantita[illegible]ely important clinical problem

The most commonly discussed pathophysiologi[illegible] mechanisms for the diarrhoea are reduced ab[illegible]ive area in connection with interrupted en[illegible] patic circulation of bile acids increased in[illegible]l secretion and increased acid load to the [illegible]rum (2 5 13 15 17) If an increased acid [illegible]y to the duodenum is an important contributor a reduction of the gastric acid secretion should improve the diarrhoeas

The aim of this pilot study was to examine whether an $H_2$ receptor blocking agent cimetidine reduces the faecal fluid losses in patients with diarrhoeas after extensive ileal resections for Crohn s disease

## PATIENTS AND METHODS

*Patients* Patients were accepted to the trial on the following conditions Ileal resections exceeding 6 dm a quiescent state of inflammatory bowel disease and at least one previous hospitalization due to dehydration and electrolyte disturbances caused by diarrhoeas and unrelated to complications or increased activity of the inflammatory disease Eleven patients (6 females) with ileal resections ranging from 7 to more than 30 dm were included 8 of the patients had ileostomy (Table I) The diagnosis of Crohn s disease according to the criteria given by Morson (14) had been verified in all by pathological examination of surgical specimens One male patient who was unable to follow instructions due to exacerbation of a paranoid psychosis dropped out and 10 patients completed the trial

The study was approved by the Ethical Committee at the Karolinska Hospital and informed consent was obtained from each subject

*Design of the study* One patient study comprised two 7-day study periods separated by an interval of 14 days (Fig 1) Eight patients were hospitalized during the study weeks and two were out patients During the study weeks the patients received a defined diet in which fat constituted less than 35% of the energy supply Using a randomized double blind and cross-over technique each patient was allocated to cimetidine 400 mg × 4 or matching placebo during days 3–7 and 24–28 of the study Cimetidine (Tagamet®) and placebo were supplied by the Smith Kline and French Laboratories Ltd England

Faeces and urine were collected on days 5 6 7 26 27 and 28 and the volumes were measured Concentrations of sodium and potassium were determined with a

Table I *Patients completing the study*

| Pat no | Sex | Age (y) | Duration of disease (y) | Years since last resection | No of resections | Length of ileal resections (dm) | Anastomosis* |
|---|---|---|---|---|---|---|---|
| 1 | ♀ | 28 | 4 | 2 | 1 | 22 | ITA |
| 2 | ♀ | 31 | 19 | 8 | 4 | >15 | IRA |
| 3 | ♂ | 30 | 9 | 4 | 1 | 18 | Ileostomy |
| 4 | ♀ | 33 | 16 | 6 | 5 | 20 | Ileostomy |
| 5 | ♀ | 29 | 16 | 2 | 8 | >15 | Ileostomy |
| 6 | ♂ | 26 | 16 | 5 | 7 | >25 | Ileostomy |
| 7 | ♂ | 40 | 15 | 1 | 2 | 7 | Ileostomy |
| 8 | ♀ | 46 | 6 | 5 | 2 | 10 | Ileostomy |
| 9ª | ♀ | 28 | 19 | 1 | 6 | 15 | Ileostomy |
| 10ª | ♂ | 33 | 22 | 11 | 3 | >30 | Ileostomy |

ª Treated as out patients
* ITA = ileotransversal anastomosis IRA = ileorectal anastomosis

flame photometric method and concentrations of fat according to van de Kamer et al (12) Blood samples withdrawn on days 7 22 and 28 were analyzed for Hb concentration WBC differential count thrombocytes reticulocytes alkaline phosphatases ASAT ALAT urea creatinine and electrolytes ($Na^+$ $K^+$ $HCO_3$ $PO_4$ Cl $Ca^{2+}$ $Mg^{2+}$) according to routine methods

*Statistics* Assuming that the measured variables were not normally distributed Wilcoxon test was used to estimate significance of differences and Spearman rank correlation test to estimate correlations

## RESULTS

The faecal losses of fluid potassium and fat during the placebo period varied widely among individuals (Table II) with a tendency to larger losses in the more extensively resected patients

Treatment with 1 600 mg cimetidine daily reduced the diarrhoeal volumes in all patients ($p<0.05$) by an average of 22% (Fig 2) As indicated by the significant positive correlation ($r=0.824$ $p<0.01$) between the placebo faecal effluent and the reduction during cimetidine treatment (Fig 3) patients with larger volume losses responded better to cimetidine The faecal sodium was reduced ($p<0.05$) in parallel by an average of 27% (Fig 2) the *reduction being positively correlated to the volume* reduction ($r=0.973$ $p<0.001$) The concentrations of sodium in the placebo collections ranged from 26 to 136 mmol $l^{-1}$ (mean 90) and did not change

Table II *Faecal volume and faecal losses of fat sodium and potassium during placebo treatment*

| Pat no | Faecal volume (ml/24 h) | Faecal fat (mmol/24 h) | Faecal sodium (mmol/24 h) | Faecal potassium (mmol/24 h) |
|---|---|---|---|---|
| 1 | 1 058 | 95 | 27 | 23 |
| 2 | 1 617 | 44 | 142 | 51 |
| 3 | 2 100 | 181 | 225 | 28 |
| 4 | 3 250 | 148 | 399 | 31 |
| 5 | 4 163 | 87 | 309 | 74 |
| 6 | 3 418 | 130 | 317 | 82 |
| 7 | 1 120 | 15 | 152 | 9 |
| 8 | 618 | 34 | 38 | 27 |
| 9 | 1 787 | 236 | 183 | 34 |
| 10 | 5 655 | 394 | 498 | 71 |

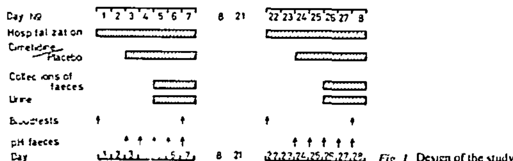



Fig 1 Design of the study

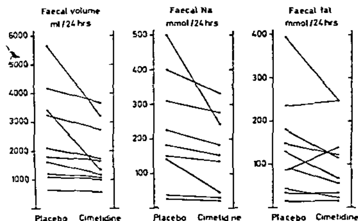



*Fig 2* Faecal volume and faecal contents of sodium and fat during placebo and cimetidine treatment. Volume reduction averaged 22% ($p<0.05$) sodium reduction averaged 27% ($p<0.05$) faecal fat change n s Each point represents the mean of collections on 3 consecutive days

significantly during the treatment period (mean 80 range 21–137) The faecal fat content decreased from control level in 6 patients increased in 2 and was unchanged in 2 (mean difference −16 range −147 to +52 mmol daily) (Fig 2) Changes in faecal potassium content were slight No significant changes were observed either in urinary volumes or in the urinary content of sodium or potassium No side-effects occurred during the treatment period and blood tests remained unchanged

## DISCUSSION

The results show that cimetidine 400 mg×4 daily significantly reduces diarrhoeal volumes and faecal sodium losses in patients operated on with extensive ileal resections for Crohn s disease Patients with the most severe diarrhoeas responded better to the treatment than patients with moderate fluid losses

No significant correlation is shown in this study between the length of ileal resection and faecal fat losses during the placebo period in contradiction to the results published by Filipsson (8) and later even by our group (11) Nevertheless our patients are selected from the group of 25 patients investigated in the latter study The absent correlation may be due to the smallness of the study population and its inhomogeneity 2 patients have parts of the colon left with an absorptive capacity that is difficult to estimate Furthermore the proportion between resected and remaining ileum is unknown especially in patients operated on at an early age

The optimal dose of a drug for patients with malabsorption is always difficult to estimate Animal studies demonstrating that cimetidine is absorbed mainly in the ileum (10) motivated the administration of 1 600 mg cimetidine daily which is a higher dose than that recommended in studies on peptic ulcer healing studies (1 4 9) Even if this

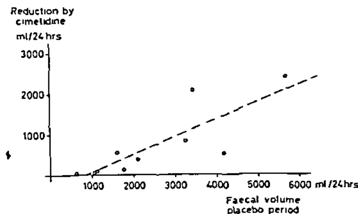



*Fig 3* Reduction of faecal volume during cimetidine treatment in relation to faecal volume during placebo period

dose was effective in reducing the diarrhoeal vol umes it may not represent the optimal dose for all patients

Existing data on gastric acid secretion after small bowel resections are conflicting (2) An increased gastric acid response to stimulation after intestinal resections (5 6 7) as well as increased plasma levels of gastrin (16) have been reported The hypothesis that the effect of cimetidine in our pa tients was due to a reduction of the gastric acid load to the duodenum is supported by a recent investiga tion (3) But other possibilities like a direct action on the transport of sodium and water through the bowel wall should also be taken into account Sec retory studies were not included in this pilot study and are, in our experience difficult to carry out in these patients who are unable to remain fasting for several morning hours The reported data suggest that cimetidine may be useful in symptomatic treatment of patients with severe diarrhoeas after extensive ileal resection Careful monitoring of plasma levels of cimetidine is advised to define the optimal dosage for each patient

## REFERENCES


1 Bodemar G Norlander, B & Walan A In Cimetidine pp 224–239 Excerpta Medica Amster dam 1977
2 Buxton B Gut 15 229 1974
3 Cortot A Fleming C R & Malagelada J R N Engl J Med 300 79 1979
4 Domschke W Domschke S & Demling L In Cimetidine pp 217–223 Excerpta Medica Amster dam 1977
5 Earnest D L Briggs F T Walsh J H & Ad mirand W H Gastroenterology 64 723 1973
6 Fielding J F & Cooke W T Gut 11 998 1970
7 Fielding J F Cooke W T & Williams J A Lancet 1 1106 1971
8 Filipsson S Malnutrition and malabsorption in Crohn s disease Thesis Göteborg 1977
9 Gillespie G Gray G R Smith I S MacKenzie I & Crean G In Cimetidine pp 240–247 Excerpta Medica Amsterdam 1977
10 Griffiths R Lee R R & Taylor D C In Cimetidine pp 38–51 Excerpta Medica Amsterdam 1977
11 Johansson C Rossner S Walldius G & Kollberg B Digestion Accepted for publication 1978
12 van de Kamer J H Ten Bokkel H & Weyers H A J Biol Chem 177 347 1949
13 Krone C H Theodor E Sleisenger M H & Jeffries G H Medicine 47 89 1968
14 Morson B C Proc R Soc Med 61 79 1968
15 Osborne M P Frederick P L Sizer J S Blair D Cole P & Thum W Ann Surg 164 622 1966
16 Strauss E Gerson S E & Yalow R S Gastroen terology 66 175 1974
17 Windsor W O Fejfar J & Woordward D A K Gut 10 779 1969

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# Acquired Angioedema and Hypocomplementemia in a Patient with Myelofibrosis

## *Effect of Danazol Treatment*

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ABSTRACT This paper reports the findings of an "acquired" hereditary angioedema like syndrome in a patient with myelofibrosis No previous personal or family history of angioedema was present The serum complement pattern showed a marked reduction of Cl esterase inhibitor, Clq and C4 All family members had a normal complement profile Because of frequent attacks of laryngeal angioedema, prophylactic treatment with danazol was started A striking clinical response was observed as well as a normalizing effect on the underlying biochemical abnormality

*Key words* danazol treatment acquired angioedema Cl *INH* deficiency hypocomplementemia, myelofibrosis

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Acquired deficiency of the early components of complement has been described in patients with *lymphoproliferative disorders and immunoglobulin* abnormalities (1 5 6 10 12) in some cases of systemic lupus erythematosus (11) and in a patient with rectal adenocarcinoma (2) Most of these patients suffered from attacks of angioedema (1, 2 5 10 12) clinically indistinguishable from hereditary angioedema (3 9) Both the acquired and the hereditary type of angioedema show a marked reduction of Cl esterase inhibitor (Cl INH) activity C4 and C2 In the hereditary type the serum level of Clq is normal whereas this has been reported to be reduced in the acquired form (1 2 5 6 10 12)

This paper describes acquired angioedema with a marked diminution of Clq Cl INH C4 and total serum hemolytic activity in a patient with myelofibrosis We also report the results of treatment with an androgen derivative danazol

## PATIENT AND METHODS

A female Caucasian aged 65 years was admitted to the Outpatient Clinic Department of Dermatology in June 1978 She had a one year history of episodic attacks of angioedema The swellings which could occur on any part of the body were tender and non pruritic The attacks were not initiated by trauma and not associated with gastric pains There was no family history of angioedema During 6 weeks prior to admission she had experienced swelling of the face lips and tongue on 3 occasions Emergency hospitalization was necessary twice because of respiratory distress The attacks subsided without treatment in 3-4 days Physical examination revealed a moderate splenomegaly otherwise unremarkable findings

Results of laboratory studies including complete blood cell count platelet count and serum chemistry screening tests were within the normal ranges Serological tests for syphilis were negative and no cold agglutinins could be detected The antinuclear antibody test was also negative Serum levels of IgG IgM and IgA were normal as was the distribution of mononuclear cell subpopulations evaluated by various surface markers (8) X ray examinations of the gastrointestinal tract lungs the spine and pelvis were normal Lymphangiography showed no filling defects of pelvic and para aortic lymph nodes Scanning of the liver and the spleen showed a moderate splenomegaly Peripheral blood showed slight anisocytosis some tear drop erythrocytes and some normoblasts Repeated bone marrow aspirations resulted in dry taps Bone marrow biopsy showed decreased cellularity with some increase of fibroblasts Spleen aspiration biopsy showed slight extramedullary hematopoiesis The diagnosis of myelofibrosis was made

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Table I *Changes in total serum hemolytic activity and complement components during treatment with danazol*

nd = Not done tr = trace amounts

| Day | Clq (%) | Total hemolytic activity (%) | Cl INH (g/l) | C4 (g/l) | C3 (g/l) | C3 proactivator (g/l) |
|---|---|---|---|---|---|---|
| 0 | 20 | 0 | 0 03 | 0 | 0 88 | 0 25 |
| 5 | 20 | 0 | 0 10 | tr | nd | nd |
| 8 | 85 | 94 | 0 15 | tr | 0 77 | 0 22 |
| 12 | 85 | 94 | 0 17 | tr | 0 85 | 0 27 |
| 15 | 70 | 80 | 0 15 | tr | 0 85 | 0 27 |
| 19 | nd | nd | 0 14 | tr | 0 81 | 0 29 |
| 22 | 25 | 20 | 0 18 | tr | 0 90 | 0 27 |
| 29 | 25 | 0 | 0 18 | tr | 0 90 | 0 32 |
| 50 | 20 | 0 | 0 15 | tr | 0 77 | 0 30 |
| 100 | 20 | 0 | 0 26 | tr | 0 85 | 0 30 |
| 150 | 20 | 0 | 0 14 | tr | nd | nd |
| Normal range | – | 90–97 | 0 15–0 40 | 0 15–0 41 | 0 6–1 28 | 0 10–0 45 |

The patient s siblings children and grandchildren had normal concentrations of the complement components

Based on the results of complement analyses of the patient s serum (vide infra) a diagnosis of acquired angioedema was made and treatment with danazol (400 mg daily) was started During an observation period of 5 months there have been no attacks of angioedema and no side-effects have been observed

*Investigational studies* C3 C4 Cl INH and C3 proactivator were quantified by single radial immunodiffusion using commercial agar plates (Behringwerke Marburg Lahn West Germany) Clq levels were also determined by radial immunodiffusion using monospecific antisera (Behringwerke) The mean level of Clq was determined from 10 normal human sera The Clq level in our patient is expressed as per cent of the normal mean The total hemolytic activity was determined as described by Kwapinski (7) Serum samples were examined before and at given intervals after initiation of the danazol treatment

## RESULTS

Repeated complement analyses of the patient s serum before treatment showed reduced levels of Cl INH Clq and C4 The concentration of C3 and C3 proactivator were within normal limits The serum showed no hemolytic activity The results of complement analyses at different intervals after treatment are summarized in Table I An increase in the Cl INH level was observed on the fifth day of treatment and normal values were found after the eighth day Trace amounts of C4 could be detected after initiation of the treatment A transient increase in Clq paralleled an increase in total hemolytic activity Nearly normal values were found on days 8 and 15 However after day 22 the concentration of Clq and the total hemolytic activity returned to values observed before treatment The treatment had no effect on the concentration of C3 and C3 proactivator

## DISCUSSION

The diagnosis of acquired angioedema in our patient was based on the clinical appearance th course of the disease and the results of complement analyses of the serum The markedly decreased concentrations of Clq Cl INH and C4 and the lack of hemolytic activity are considered sufficient to exclude a hereditary defect (5) In addition normal values of the various complement components were found in the sera of the patient s siblings children and grandchildren In contrast to previously reported cases (1 5 6 10 12) we did not detect any abnormalities in the immunoglobulins or any neoplastic disease in our patient

Treatment with danazol an ethinyltestosterone analogue with a pituitary antigonadotrophin effect has given excellent protection against angioedema of the hereditary type (4) Relatively small doses (300–600 mg a day) normalized the values of Cl INH and C4 in most of the patients Even without

achieving complete normalization of the complement components danazol seemed to prevent clinical manifestations of hereditary angioedema

Danazol treatment has not been studied as thoroughly in cases of acquired angioedema Cohen et al (2) observed a return to normal levels of all complement components in a patient with acquired angioedema complement deficiencies and rectal adenocarcinoma Hauptmann et al (6) found that C1 INH reached normal values while C4 remained low in a patient with a lymphoproliferative disorder and deficiencies of the early complement components but apparently not angioedema Consistent with these findings we observed a rapid normalization of the C1 INH concentration which remained within normal limits during the observation period We did not detect any increase in the concentration of C4 which is in line with the results reported by Hauptmann et al (6) In contrast Cohen et al (2) found that danazol normalized the concentration of C4 but after reducing the dose all the complement components returned to pretreatment levels and increasing the danazol dose did not influence these values Something similar was observed in our patient in whom C1q and serum total hemolytic activity subsequently to a significant rise returned to decreased levels after 3 weeks of treatment

Our study shows that acquired deficiency of the early complement components and attacks of angioedema may also occur in patients without neoplastic disorders It also supports the suggestion by Hauptmann et al that danazol may effectively prevent attacks of acquired angioedema (6) The most constant effect of danazol on the complement system both in the hereditary and the acquired type seems to be the elevation of the C1 INH level It is therefore reasonable to assume a connection between the normalization of C1 INH and the prevention of angioedema in both conditions The precise mechanisms regarding the effect of danazol on the various components of complement still remain to be clarified


## ACKNOWLEDGEMENT

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## REFERENCES


1 Caldwell J R Ruddy S Schur P H & Austen K F Acquired C1 inhibitor deficiency in lymphosarcoma Clin Immunol Immunopathol 1 39 1972
2 Cohen S H Koethe S M Kozin F Rodey G Arkins J A & Sink J N Acquired angioedema associated with rectal carcinoma and its response to danazol therapy J Allergy Clin Immunol 62 (4) 217 1978
3 Donaldson V H & Evans R R A biochemical abnormality in hereditary angioneurotic edema absence of serum inhibitor of C1-esterase Am J Med 35 37 1963
4 Gelfand J A Sherin R J Alling D W & Frank M M Treatment of hereditary angioedema with danazol Reversal of clinical and biochemical abnormalities N Engl J Med 295 (26) 1444 1976
5 Hauptmann G Lang J M North M L Oberling F Mayer G & Lachmann P Acquired C1 inhibitor deficiencies in lymphoproliferative diseases with serum immunoglobulin abnormalities Blut 32 195 1976
6 Hauptmann G Mayer S Lang J M Oberling F & Mayer G Treatment of acquired C1 inhibitor deficiency with danazol Ann Intern Med 87 (5) 577 1977
7 Kwapinski J B Methods of serological research Wiley New York London and Sydney 1965
8 Matre R Talstad I & Haugen Å Surface markers in non phagocytic hairy cell leukemia Acta Pathol Microbiol Scand (C) 85 406 1977
9 Rosen F S Charache P Pensky J & Donaldson V H Hereditary angioneurotic edema. two genetic variants Science 148 957 1965
10 Rosenfeld S I Staples P J & Leddy J P Angioedema and hypocomplementemia. Unusual features of lymphoma J Allergy Clin Immunol 55 104 1975
11 Ruddy S & Austen K F Natural control mechanisms of the complement system In Biological activities of complement (ed D C Ingram) p 13 Karger Basle 1972
12 Schreiber A D Zwiman B Atkins P et al Acquired angioedema with lymphoproliferative disorder Association of C1 inhibitor deficiency with cellular abnormality Blood 48 (4) 567 1976

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# Effect of Cyclofenil Treatment on Arterial Insufficiency Demonstrated in a Patient by Colour Thermography

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ABSTRACT Cyclofenil, 200 mg t i d , was administered for four months to a 67 year-old woman, who suffered from a combination of scleroderma, Osler–Weber–Rendu disease and a severe atherosclerotic circulatory insufficiency The effects on the severely impaired skin circulation in the face and hands were followed and recorded by colour isothermograms, using the AGA monitor system The treatment resulted in a marked improvement of the arterial circulation with disappearance of the Raynaud phenomenon complete arrest of gastrointestinal bleeding, disappearance of malabsorption, and relief of the joint stiffness

*Key words* vasodilating drug arterial insufficiency cyclofenil—non-oestrogenic derivative scleroderma colour thermography

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It has been previously reported from animal experiments that cyclofenil a compound with very weak oestrogenic and some anti oestrogenic properties inhibits synthesis of chondro-mucopolysaccharides (4 5 6 7) In order to ascertain the clinical effects of cyclofenil a number of sclerodermatous patients were studied There was a marked improvement in the skin condition joint and muscle rigidity respiratory function and peripheral circulation (8 9 10)

The promising effect of cyclofenil on the Raynaud phenomenon in our patients led us to investigate the peripheral circulation before and at certain times after starting cyclofenil treatment For this purpose we used a colour thermographic method which had been previously improved (1 2 3) Our patient had an advanced sclerodermatous state together with the Osler–Weber–Rendu syndrome and also a marked atherosclerotic circulatory insufficiency with a history of several episodes of gangrene in the toes and fingers

## THE THERMOGRAPHIC METHOD

Thermography has been developed during the last years into a powerful tool for assessing the arterial circulatory conditions in different regions of the body An AGA thermovision colour monitor (AGA Sweden) was employed to measure the infrared radiation from the skin and subcutaneous tissues of the face and hands The results were evaluated with the colour thermographic technique

The AGA apparatus was equipped with 8 colour filters thus allowing the simultaneous recording of 8 isotherms (IT) The skin surface was first cleaned and the thermograms were made in a room maintained at a constant temperature (22°C) and relative humidity (45%) The filters were chosen to give the ITs with 1 C differences Normally in the face the warmest patch is located in the medial corner of the eye and in the hands over the radial artery at the wrist These hot spots were used as standard values for IT calibration

All thermograms were stored in the memory bank of a computer until processing The colour ITs were standardized as follows 1) White IT 0 85–0 8 2) Yellow IT 0 8–0 75 3) Red IT 0 75–0 7 4) Lilac IT 0 7–0 65 5) Light green IT 0 65–0 6 6) Dark green IT 0 6–0 55 7) Light blue IT 0.55–0 5 8) Dark blue IT 0.5–0 45 The temperature resolution is thus 1 C over an 8°C range If a region had a temperature lower than the selected range it was necessary to use a new filter The range was then shifted to lower IT values These were recorded with the same colour scheme as before In this way it was thus possible to record 16 ITs

## CASE REPORT

The patient who was under the care of M Martini and P Tabak was a 67 year-old house wife without any family history of connective tissue or vascular disease Her two sons were healthy and she was a non smoker

Her present illness began when she was about 45 with signs of morphoea which started with cutaneous telangiectasia of the Osler type and continued with relapsing gastrointestinal telangiectases which gave rise to severe abdominal pains haematemesis and melaena When she was 48 a scleroderma appeared and developed progressively At the age of 53 Raynaud like signs appeared especially in her arms and hands with severe impairment

Table I *Some laboratory data before and after cyclofenil treatment*

| | Before treatment | After 4 months of treatment |
|---|---|---|
| ESR (mm/h) | 28 | 20 |
| Hb (g/100 ml) | 7 2 | 10 0 |
| Leukocytes | 4 400 | 4 600 |
| Stool Weber | ++ pos – +++ pos | Neg – Neg |
| *Tests of liver function* | | |
| Serum bilirubin (mg/100 ml) | 0 66 | 0 50 |
| Thymol (U) | 1 4 | 2 3 |
| Mollea | Neg | Neg |
| Bromsulphalein 45 | Neg | Neg |
| S-ALP (IU) | 103 | 99 |
| S-GPT (S-ALAT) (IU) | 26 | 23 |
| S-GOT (S-ASAT) (IU) | 77 | 99 |
| Serum total proteins (g/100 ml) | 6 5 | 6 4 |
| *Electrophoresis* | | |
| Albumin (%) | 50 | 40 |
| Globulins (%) | 3 | 6 |
| | 10 | 11 |
| | 14 | 18 |
| | 23 | 25 |
| Serum creatinine (mg/100 ml) | 0 78 | 0 62 |
| Plasma urea (mg/100 ml) | 20 8 | 13 2 |
| Serum glucose (mg/100 ml) | 85 | 77 |
| Serum total lipids (mg/100 ml) | 980 | 760 |
| Serum cholesterol (mg/100 ml) | 260 | 220 |
| Pulmonary vital capacity (ml/%) | 1 200/46 | 1 350/50 |

of the arterial circulation Temporary relief was obtained after large doses of nicotinic acid and vitamin E However the main physical signs including severe melaena continued and she was given blood transfusions on 18 occasions At the age of 57 she developed a rapidly progressive oesophageal constriction with a stenosis just above the cardia. She then had to be tube fed a liquid and mushy diet During this period there was a gradual loss of weight leading to cachexia

The persistent symptoms were treated from time to time with high doses of nicotinic acid vitamin E prednisolone and penicillamine These drugs caused several side effects without any noticeable improvement Pharmacological evaluation of these drugs and other compounds tested later has been published elsewhere (9)

When the patient was 64 she developed a progressive obliterative atherosclerosis in her lower limbs first on the right side and later on the left Marked gangrene with severe pain occurred in toes I and II of her right foot The circulatory insufficiency together with the contractions of her knee joints and a severe muscle dystrophy made it impossible for her to walk and she could only sit or lie down One year later it was necessary to perform right and left sympathectomy These operations neither relieved the pain nor did they influence the severe gangrene Repeated infusions of Rheomacrodex® (Pharmacia Sweden) were also tried without success

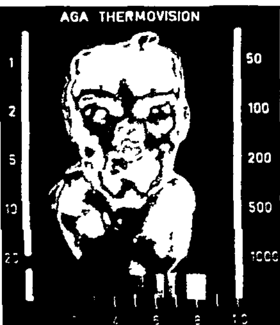
After extensive X ray examination it was decided to amputate her right leg at the mid femoral level A thorough histopathological examination of skin specimens from the amputated limb yielded the following results microscopic sections of the skin showed atrophy of epidermal layers flattening of the papillae and of the rete pegs All epidermal appendages had disappeared Beneath the epidermis there were tight broken and slightly basophilic streaks and dense collagen fibres with thick streaks These histopathological findings supported the diagnosis of severe sclerodermatous disease with necrotizing arteritis

The clinical condition of the patient deteriorated week by week after the amputation Her left lower limb had a 90° contracture of the knee joint and she could only move herself in a wheel-chair There was severe pain in the whole of her left leg and gangrene developed in the tip of the fourth toe She required strong analgesics daily Because of the severely impaired circulation amputation of her left lower limb was also considered but she refused and became severely depressed Oral treatment with cyclofenil (non-oestrogenic drug) was then started in a dose of 200 mg t i d and continued for 4 months during which period her progress was checked with laboratory tests and thermographic investigations The laboratory results are collected in Table I and the thermographic data together with her subjective feelings are presented below

## RESULTS

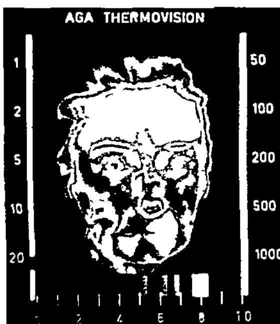
### *Clinical improvements*

The patient felt well almost immediately after the beginning of cyclofenil treatment and already after

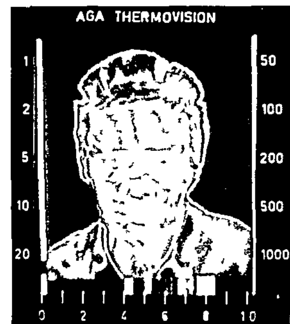



a

*Fig 1* Circulatory conditions in the face before treatment (*a*) after one month (*b*) and after four months of daily cyclofenil administration (*c*) The temperature dependent isotherms are visualized with colour thermography




b




c







a week her mental depression had disappeared Characteristic warm sensations were felt in the face and in all limbs There was also a marked relief of the severe leg and toe pains and she no longer required strong analgesics It also became much easier to swallow food and after four months she was able to eat and swallow normally and gained 2 kg in weight From this period on she did not need the wheel chair and could walk with a prosthesis and a stick The severe gangrene on the tip of the left fourth toe which was previously purulently infected became dry and well demarcated After one year the whole would was completely healed

*Colour thermographic studies*

Fig 1 shows colour ITs of the face before one month and four months after the beginning of cyclofenil treatment It can be seen that there was a rapid and progressive elevation in the skin and subcutaneous temperature signifying circulatory improvement After one month the circulation had already improved and the whole forehead and the tip of the nose became 1 C warmer After four months the temperature had increased markedly in the whole left half of the face

Fig 2 shows colour ITs of the hands There was a marked improvement of the circulation in both hands The changes were much more prominent than those in the face tissues (Fig 1) and extended even to the tips of the fingers In the pretreatment condition the distal parts of the fingers were 11 C colder than the standard hot areas over the wrists After one month s treatment the palmar regions became 3–4 C warmer and the fingers became about 7 C warmer than previously After four months treatment (Fig 2*c*) both hands showed a very intense temperature increase

## DISCUSSION AND CONCLUSIONS

This patient suffered from a complex disease involving many different processes The whole clinical picture began with morphoea with round patches of violet hard skin Later a classical Raynaud phenomenon developed in the upper and lower limbs followed by atrophy of the skin and subcutaneous tissues The condition developed into an Osler–Weber–Rendu like syndrome with obvious cutaneous telangiectases together with gastrointestinal telangiectasic areas which caused very severe intestinal bleeding with several episodes of melaena and haematemesis Additionally she developed a severe iron deficiency anaemia A Henoch–Schonlein purpura was considered as an alternative diagnosis The classical features of this condition include gastrointestinal complications with intramural haematomas and severe mucosal haemorrhages The colicky pains haematemesis and melaena fit in better with the Osler–Weber–Rendu disease Moreover she also had a classical scleroderma and a very severe atherosclerotic condition with extremely severe consequences If we compare the clinical status before and after four months of cyclofenil administration it is obvious that there was a marked therapeutic effect The profound circulatory improvement could be demonstrated quite unequivocally by the thermographic recordings It is evident that only four months long treatment resulted in a roughly 6 C increase of the temperature in the tissues investigated This rapidly appearing vasodilating effect of cyclofenil is in good agreement with our previously observed general amelioration in cases of scleroderma (8 10)

This improved thermographic technique could probably be utilized in several conditions with impaired vascular function such as atherosclerosis thromboangiitis obliterans the Raynaud syndrome and possibly also in diabetic gangrene

## REFERENCES


1 Boczan J Electroencephalographic and thermographic examinations during pharmaco-therapy in catatonic schizophrenia Electroencephalogr Clin Neurophysiol 32 458 1972
2 — Effect of different climatic conditions on patients with catatonic schizophrenia and hyperthyroidism Int J Med Geogr 4 46 1973
3 — Thermographische Messungen an Gesicht Hand und Unterschenkel vor und nach Gabe von Bencyclan Folia Angiol 23 454 1975
4 Herbai G Effect of age sex starvation hypophysectomy and growth hormone from several species on the inorganic sulphate pool and on the incorporation in vivo of sulphate into mouse costal cartilage An attempt to study sulphation factor activity in vivo Acta Endocrinol (Kbh) 66 333 1971
5 — Effect of pregnancy castration testosterone ethisterone oestradiol benzoate and stilboestrol on the exchangeable sulphate pool and on sulphate incorporation in vivo into costal cartilage of the mouse Acta Pharmacol Toxicol (Kbh) 29 177 1971
6 — Separation of growth inhibiting potency from oestrogenicity in different weak oestrogenic drugs of various chemical structures Acta Endocrinol (Kbh) 68 249 1971

7 — Studies on the site and mechanism of action of the growth inhibiting effects of estrogens Acta Physiol Scand 83 77, 1971
8 — Treatment of progressive systemic sclerosis with a synthetic weak estrogen cyclofenil (Sexovid®) Acta Med Scand 196 537 1974
9 — Scleroderma (progressive systemic sclerosis PSS) Pathophysiological clinical and pharmacological aspects of the syndrome Acta Med Acad Sci Hung 35 201 1978
10 Herbai G Blom B & Bostrom H Treatment of progressive systemic sclerosis (scleroderma PSS) with a new drug influencing connective tissue Acta Med Scand 201 203 1977

# Reveısıble Bone Marrow Granulomas—Adverse Effect of Oxyphenbutazone Therapy

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ABSTRACT A 48 year old woman treated with oxyphenbutazone developed fever, gastrointestinal disturbances, mucocutaneous reactions, leukopenia, eosinophilia and thrombocytopenia Bone marrow biopsy showed granulomatous lesions Following withdrawal of the drug, all signs and symptoms subsided and the blood changes and the bone marrow biopsy normalized The granulomatous reaction in the bone marrow is considered to be a hypersensitivity manifestation of oxyphenbutazone

*Key words* adverse effect oxyphenbutazone bone marrow granuloma leukopenia thrombocytopenia

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There are many reports on the adverse effects of oxyphenbutazone and phenylbutazone therapy The most frequently reported side effects are gastrointestinal disturbances edema and mucocutaneous reactions (7 9) Variable degrees of bone marrow depression may occasionally develop (1 3) Also a number of reports describe granulomatous inflammation in the liver associated with these medications (2 4 5 6)

In this report we present a case with several adverse effects associated with oxyphenbutazone therapy One of them was granulomatous inflammation in the bone marrow which disappeared after termination of oxyphenbutazone therapy

## CASE REPORT

A 48-year old woman previously healthy except for episodes of pain and stiffness in her right shoulder was given oxyphenbutazone (Tanderil®) 200 mg three times a day for progressive shoulder pain She received no other medications

On the second day of therapy she developed abdominal pain diarrhea and skin rash During the following two days fever and a sore throat were also noted Following four days of oxyphenbutazone therapy (total of 2 4 g) she was admitted to hospital and oxyphenbutazone was discontinued Physical examination on admission revealed a maculopapular rash over the trunk and limbs Small ulcerations were seen in the throat The liver spleen and lymph nodes were not enlarged The rectal temperature was 38 8°C

Laboratory data on the day of admission Hb 112 g/l Hct 32% platelets $100\times10^9$/l WBC 3 $2\times10^9$/l with 33 5% band neutrophils 36 5% segmented neutrophils 10 5% eosinophils 17 0% lymphocytes and 2 5% monocytes The serum transaminases bilirubin alkaline phosphatase electrolytes and creatinine were within normal limits Bacterial cultures of the blood urine and nasopharynx were all negative A chest roentgenogram was normal

A bone marrow biopsy specimen demonstrated multiple small ill-defined and occasionally confluent granulomas These granulomas contained epithelioid histiocytes and small numbers of lymphocytes and plasma cells (Fig 1) The reticulin stain (Gordon Sweet) showed a slightly increased quantity of reticulin within the granulomas No relationship between granulomas and blood vessels was identified and no vasculitis was seen The special stain for acid fast bacilli was negative

The overall marrow cellularity was within normal limits for a patient of this age and no lymphoid aggregates were present There was active granulopoiesis and all stages of myeloid maturation were represented however a left shift in the granulocytic maturation was noted The granulocytopoietic maturation ratio was 1 2 2 Myeloblasts constituted less than 1% of the nucleated marrow cells and there was a slight increase in the number of eosinophils

Erythropoiesis was normoblastic and the myeloid/erythroid ratio (M/E) was 6 1/1 Neither the lymphocytes nor the plasma cells were increased in number and no granulomatous tissue was present in the marrow aspirate smears Stains for acid fast bacilli were negative

Although no medical treatment—other than isolation from other patients in the ward—was instituted the diarrhea and abdominal pain rapidly disappeared and the skin rash faded After five days her blood cell count had normalized and on the sixth day she was discharged in good condition Repeat blood cell counts 3 and 8 weeks after discharge were normal Furthermore bone marrow biopsies performed one and three months after discharge showed no granulomas and a normal marrow histology was seen with normalized M/E and granulocytopoietic

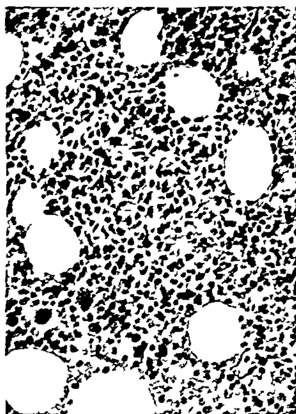


*Fig 1* Bone marrow showing a small granuloma Hematoxylin and eosin ×205

maturation ratios Roentgenograms of her chest and right shoulder disclosed no abnormalities

## DISCUSSION

Many drugs and chemicals are believed to cause granulomatous reactions in human tissues Two such drugs are phenylbutazone and oxyphenbutazone Oxyphenbutazone is a hydroxylated ring metabolite of phenylbutazone and these two drugs have similar pharmacological and toxicological properties (7)

Eleven cases to date have been reported in which patients developed granulomas in various organs due to phenylbutazone or oxyphenbutazone therapy Ishak et al (4) reviewed these cases and found that the duration of therapy varied from 8 to 42 days The amount of phenylbutazone ingested prior to the onset of the patients illness varied from 3 6 to 70 0 g (average 15 8) while that for the two patients on oxyphenbutazone was 1 8 and 2 1 g (average 1 95)

Granulomas were found in the liver (11 cases) and in a lymph node (1 case) Widespread granulomas including bone marrow granulomas were seen in one fatal case (6) The granulomatous inflammation in these cases was considered to be part of a general hypersensitivity reaction Other manifestations of hypersensitivity such as fever skin rash hepatic injury and lymphadenopathy were also seen

Bone marrow granulomas of the same type as in our patient have been described by Rywlin (8) in association with chlorpropamide allopurinol and procainamide therapy The granulomatous inflammation seen in association with such medications may resemble the granulomas found in Hodgkin s disease tuberculosis and sarcoidosis

The clinical manifestations and the bone marrow changes in our patient were considered from the very beginning to be adverse effects of oxyphenbutazone Moreover all the clinical manifestations blood changes and granulomatous lesions in the bone marrow disappeared following termination of the oxyphenbutazone therapy In contrast to the cases reviewed by Ishak et al (4), our patient received no other medication than oxyphenbutazone and she was healthy except for her shoulder pain Though the sensitization period in our patient was brief she did have several manifestations of generalized hypersensitivity which have previously been reported in association with oxyphenbutazone therapy including fever, rash stomatitis and eosinophilia (7) The granulomas found in the bone marrow of our patient were of the same type as those previously seen in association with other drugs and considered to be of hypersensitive origin

We therefore consider the granulomatous reaction in the bone marrow of our patient to be a hypersensitivity manifestation of oxyphenbutazone To the best of our knowledge this is the first reported case of reversible bone marrow granulomas associated with oxyphenbutazone therapy

## REFERENCES


1 Bottiger L E & Westerholm B Drug induced blood dyscrasias in Sweden Br Med J 3 339 1973
2 Goldstein G Sarcoid reaction associated with phenylbutazone hypersensivity Ann Intern Med 59 97 1963
3 Inman W H W Study of fatal bone marrow depression with special reference to phenylbutazone and oxyphenbutazone Br Med J 1 1500 1972
4 Ishak K G Kirchner J P & Dhar J K Granulomas and cholestatic hepatocellular injury as

sociated with phenylbutazone Digestive Diseases 2 611 1977
5 Lundqvist M Alcoholic cirrhosis and other toxic hepatopathies In Alcoholic cirrhosis Scandia International Symposia (ed A Engel & T Larsson) pp 249–250 Nordiska Bokhandelns förlag Stockholm 1970
6 O Brien D J Death from hypersensitivity due to phenylbutazone Br Med J 1 792 1954
7 Prescott L F Meyler s side effect of drugs A survey of unwanted effects of drugs reported in 1972–1975 (ed M N G Dukes) pp 211–214 Excerpta Medica Elsevier New York 1976
8 Rywlin A M Histopathology of the bone marrow p 176 Little Brown & Co Boston 1976
9 Sperling I L Adverse reactions with long term use of phenylbutazone and oxyphenbutazone Lancet 2 535 1969

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# Minor Signs and Symptoms of Toxicity in a Young Woman in Spite of Massive Thyroxine Ingestion

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ABSTRACT The serum concentrations of thyroxine, 3,5,3 triiodothyronine and 3,3',5 triiodothyronine were followed during nine days in a case of acute thyroxine intoxication On admission the concentrations were 11–12 times, 5–6 times and 16 times the normal mean, respectively There was a striking discrepancy between the high concentrations of active hormones and the minor clinical symptoms

*Key words* intoxication thyroxine suicide

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The most common thyroid disorders demanding thyroid hormone substitution treatment are autoimmune hypothyroidism Graves disease (after operation or radioiodine treatment) and endemic goiter The number of patients is not small—during a population survey of middle aged females in Gothenburg we found that about 4% of the women received thyroxine ($T_4$) or related drugs Despite the common use of such drugs acute intoxications are uncommon In published reports the symptoms have usually been mild but in a few cases the patients have been seriously ill (1 4–7 9) Information regarding thyroid hormone concentrations have been scarce

The present report deals with a young woman with acute $T_4$ intoxication The concentrations of $T_4$ 3 5 3 triiodothyronine ($T_3$) and 3 3 5 triiodothyronine (reverse $T_3$ $rT_3$) were followed during the course of intoxication The case is of interest as it shows a discrepancy between the serum concentrations of active thyroid hormone(s) and their metabolic effects in the acute phase of an intoxication

## CASE REPORT

A 19 year-old female student was admitted to the hospital 16 hours after an overdose of more than 100 tablets of levothyroxine (Levaxin® 0 1 mg Nyegaard Oslo Norway) For two years she had been taking $T_4$ because of autoimmune hypothyroidism with goiter and had responded well She had a history of yearly mental depressions and had presently had problems with her studies She experienced no discomfort apart from slight tachycardia On examination she appeared untroubled with only slight hand tremor The skin was somewhat warm and moist and the pulse rate was 120/min otherwise physical examination gave normal results The blood pressure was 120/80 mmHg No glucosuria or albuminuria were found The rectal morning temperature which was 36 7°C rose to 37 8°C during the following days and was normal from day 4

Determinations of serum tri and tetraiodothyronine by radioimmunoassay (2) showed very high concentrations (Fig 1) The concentration of serum $T_4$ was 1 460 nmol/l on admission (normal range 80–170) The concentration of $T_3$ was 11 6 nmol/l (normal range 1 5–3 0) and that of $rT_3$ 5 6 nmol/l (normal range 0 14–0 55)

The patient received propranolol 20 mg×3 during days 1–4 and her pulse rate was then around 100/min On day 5 the pulse rate rose to 120/min and the propranolol dose was increased to 40 mg×3 The clinical course was uneventful As shown by Fig 1 the serum concentrations of $T_4$ $T_3$ and $rT_3$ decreased but were still abnormally high when the patient was discharged after 9 days

## DISCUSSION

The discrepancy between the laboratory and clinical findings in our patient was striking She had no significant thyrotoxic symptoms on admission in spite of the high concentration of $T_3$ 5–6 times the normal mean and $T_4$ 11–12 times the normal mean The concentration of $rT_3$ was about 16 times the normal mean Thyrotoxic patients with such high concentrations of $T_3$ and $T_4$ usually have pronounced symptoms Possibly the duration of exposure to high thyroid hormone concentrations is of importance in the development of the signs and

*Abbreviations* $T_4$=thyroxine $T_3$=3 5 3 triiodothyronine $rT_3$=3 3 ,5 triiodothyronine

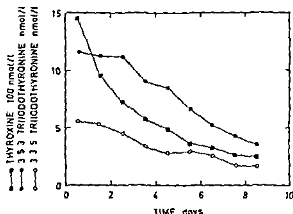



*Fig 1* Serum concentrations of thyroxine 3,5 3 triiodo thyronine and 3 3 ,5 triiodothyronine ( reverse triiodo thyronine) in a case of acute thyroxine intoxication

symptoms of toxicity One may also speculate on the possibility that the dramatic increase in $rT_3$ concentration may play a role in inhibiting the metabolic effects of the active thyroid hormone(s) The possibility of such effects has been shown albeit with larger doses of $rT_3$ but results from studies in man tend to refute these actions as physiological phenomena (ref 8 and its references)

The apparent $t\frac{1}{2}$ for the elimination of $T_4$ from the blood was 3 days during treatment with the low dose of propranolol and 5 days with the high dose These values which are lower than those found in normal individuals are in good agreement with observations by other authors that $T_4$ affects its own metabolism (3) and that $T_4$ monodeiodination may be inhibited by propranolol (10)

The finding of a relatively larger increase in the concentration of $rT_3$ than of $T_3$ indicates a relative block of the deiodination of the outer ring in $T_4$ or a preferential deiodination of the inner ring with an attempt by the body to reduce the formation of active thyroid hormone from $T_4$ This conforms with the general opinion on the biological role of the two pathways for $T_4$ deiodination

As there was no correlation between serum thyroid hormone concentrations and the signs of toxicity there is apparently no need for emergency testing of serum thyroid hormones and treatment of acute $T_4$ intoxication should be based on the clinical findings

## REFERENCES


1 Bakkers F J M & van der Does E Thyroxine in toxicatie Ned Tijdschr Geneeskd 116 880 1972
2 Blomquist N Lindstedt G Lundberg P A & Wålinder J No inhibition by $Li^+$ of thyroxine monodeiodination to 3 5 3 triiodothyronine and 3 3 5 triiodothyronine (reverse triiodothyronine) Clin Chim Acta 79 457 1977
3 Braverman L E Vagenakis A Downs P Foster A E Sterling K & Ingbar S H Effects of replacement doses of sodium l thyroxine on the peripheral metabolism of thyroxine and triiodothyronine in man J Clin Invest 52 1010 1973
4 Funderburk S J & Spaulding J S Sodium levothyroxine (Synthroid R) intoxication in a child Pediatrics 45 298 1970
5 Goulon M & Combes, A Crise thyrotoxique medicamenteuse Nouv Presse Med 6 3729 1977
6 Hempel R D & Burchardt U Akute Intoxikation mit 1 Trijodthyronin 1 Thyroxin and Phendimetrazinbitartrat aus suizidaler Absicht Z Gesamte Inn Med 31 296 1976
7 Levy R P & Gilger W G Acute thyroid poisoning N Engl J Med 256 459 1957
8 Nicod P Burger A Strauch G Vagenakis A G & Braverman L E The failure of physiologic doses of reverse $T_3$ to affect thyroid pituitary function in man J Clin Endocrinol Metab 43 478 1976
9 Schottstaedt E S & Smoller M Thyroid storm produced by acute thyroid hormone poisoning Ann Intern Med 64 847 1966
10 Verhoeven R P Visser T J Docter R Hennemann G & Schalekamp M A D H Plasma thyroxine 3 3 5 triiodothyronine and 3 3 5 triiodothyronine during β adrenergic blockade in hyperthyroidism J Clin Endocrinol Metab 44 1002 1977

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# Nonsecretory Myeloma Associated with Nodular Glomerulosclerosis

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ABSTRACT. A patient with nonsecretory multiple myeloma in association with nodular glomerulosclerosis is reported. The clinical course was characterized by rapidly progressing renal insufficiency terminating in uremia within three months. Histological investigation of the kidney revealed extensive nodular formations in the mesangial areas consisting of basement membrane-like material, in places with content of fibrils. Immunofluorescence demonstrated deposition of kappa chains. Amyloid was not present. Routine methods for investigation of serum and urine showed severe hypogammaglobulinemia without M components. Using a sensitive radioimmunoassay for free light chains, an abnormal non dissociable kappa chain polymer with a molecular weight of 55000 was found in serum and urine. The findings support the hypothesis that mesangial accumulation of paraproteins induce an increased synthesis of basement membrane material leading to formation of the nodules. Abnormal polymerization of kappa chains might provoke the formation of the glomerular nodules.

*Key words:* electron microscopy, immunoglobulins, light chains, kidney, nodular glomerulosclerosis, nonsecretory myeloma.

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Nodular glomerulosclerosis, similar to the lesion seen in diabetes mellitus, has occasionally been described in association with multiple myeloma. A completely normal glucose tolerance test is seen in these patients. The clinical course is characterized by rapidly progressing renal failure. Deposition in the glomerulus of the M component is thought to induce the formation of the nodules (3, 5, 13). In about 1 % of the patients with multiple myeloma no M-component is found in serum or urine (15). These cases are denoted as nonsecretory myeloma.

We report our findings in a patient with nonsecretory myeloma in association with the characteristic glomerular nodular lesion. Only by a sensitive radioimmunoassay for light chains was an increased concentration of kappa chains found in serum and urine. The kappa chains were found to possess a highly abnormal polymerization property.

## CASE REPORT

The patient, a 61 year-old man, had formerly been in good health. Initial symptoms were nausea, vomitings and after a few days abdominal pains and diarrhea. A general practitioner discovered microscopic hematuria and the patient was admitted to hospital.

Physical examination revealed malignant hypertension with a blood pressure of 240/130 mmHg and ophthalmoscopy showed papillary edema, hemorrhages, exudates and severe arteriolar changes. Lymph nodes, spleen or liver were not enlarged. After treatment with diuretics blood pressure normalized and remained normal throughout the course. During hospitalization the patient's condition was characterized by periodic confusion. Renal insufficiency with creatinine clearance of 25–35 ml/min was found on admission. His renal function deteriorated progressively, and he died in uremia 3 months after onset of the first symptoms.

The patient was treated with melphalan and prednisone without noticeable effect. Laboratory investigations revealed a normochromic anemia with Hb concentration of 10.7 g/100 ml. WBC decreased from 15000 cells/μl to normal values after a few days. The differential counts were normal and no immature cells or plasma cells were seen. The thrombocyte counts were also normal. ESR was 30–67 mm/h. S-uric acid was elevated to approximately 15 mg/100 ml. Paper electrophoresis and immunoelectrophoresis of the serum proteins showed a total gammaglobulin concentration of 0.2 g/100 ml (normal 0.9–1.5) with no evidence of monoclonal components. S-albumin was decreased to approximately 2.9 g/100 ml (normal 3.6–5.0). S-Ca was elevated to 14 mg/100 ml on admission and normalized after institution of treatment with prednisone. The concentration of alkaline phosphatase was normal on admission but slightly elevated during the last month. Parathyroid hormone levels were normal. X ray examination of the skeleton showed no

Table I *Concentrations of IgG IgA and IgM (g/l)*

| | Patient s values | | Normal range |
|---|---|---|---|
| | Mean | Range | |
| IgG | 2 03 | 1 32–3 36 | 6 30–13 25 |
| IgA | 0 57 | 0 22–0 76 | 0 85– 3 80 |
| IgM | 0 10 | 0 08–0 10 | 0 15– 1 00 |

osteolytic lesions Halisteresis of the skull was however seen Blood sugar values were normal no glucosuria was seen and the oral glucose tolerance test was normal Urine investigation (performed by a sulfosalicylic acid precipitation technique) revealed severe proteinuria of 6–10 g/24 h Electrophoresis on cellulose acetate membranes (Microzone technique Beckman[a]) showed proteinuria of non selective glomerular pattern with a urinary protein pattern similar to serum The electrophoresis failed to demonstrate any monoclonal immunoglobulins Histological investigation of the bone marrow showed 40% plasma cells with many immature cells

## METHODS

*Quantitation of immunoglobulins* IgG IgA and IgM were estimated once a week by a modification of the rocket immunoelectrophoresis (10) and IgD by immunodiffusion in agarose gel (LC Partigen plates Behringwerke detection limit approximately 0 04 of British Research Standard no 67/37) IgE was estimated by radioimmunoassay (Medicinsk Laboratorium Denmark detection limit 10 U=approximately 20 μg/l)

*Free light chains* in serum and urine were measured by radioimmunoassay (22 23) The samples were filtered on Sephadex G 100 SF columns (140×1 5 cm) in order to separate the free light chains from regular immunoglobulins Non absorbed broadly reactive antibodies against kappa and lambda chains could be used for this reason

*Circulating immune complexes* in serum were measured by radioimmunoassay using a C1q binding technique (21) and by measuring light chain determinants in immune complexes precipitated by 2 5% PEG (20)

*Histological methods*

*Renal tissue* For light microscopy the renal biopsy was fixed in Carnoy s fluid and 2 μm serial paraffin sectic is were stained with hematoxylin-eosin periodic acid-Schiff periodic acid silver methenamine and picro-Sirius red The phosphotungstic acid hematoxylin reaction was used for detection of fibrin and for amyloid the alkaline Congo red methyl violet and Sirius red F3B reactions were employed Immunofluorescence technique was performed on fresh frozen sections of biopsy material as described earlier (14) For electron microscopy small pieces of biopsy material were fixed in 2 5% cacodylate buffered glutaraldehyde (pH 7 3) for one hour and postfixed in 1% $OsO_4$ for one hour After dehydration in ethanol and embedding in Vestopal ultrathin sections were cut on an LKB ultramicrotome Sections were mounted on Formvar coated copper grids and stained with uranyl acetate and lead citrate Specimens were examined with a Philips EM 201 electron microscope

*Bone marrow* Intracellular accumulation of immunoglobulins in the plasma cells was investigated by the immunoperoxidase technique (25) Antibodies against kappa lambda gamma alpha and mu chains were used

## RESULTS

*Immunological studies of serum and urine*

The concentrations of IgG IgA and IgM compared with normal ranges are shown in Table I All three classes of immunoglobulins showed depressed values

The concentrations of IgD and IgE were below the detection limit Table II shows the serum concentrations of the polymeric forms of kappa and lambda chains The total concentration of kappa chains is increased also when compared with values of patients with similar decreased renal function (23) The table shows that the increased concentration of kappa chains is mostly due to an abnormal protein (see below) The concentrations of kappa chain monomers and lambda chains are de

Table II *Concentrations of polymeric forms of kappa and lambda chains in serum (mg/l) compared with normal values (mean ± S D )*

| | Abnormal protein | Dimers | Monomers | Total concentration |
|---|---|---|---|---|
| Kappa chains | | | | |
| Patient s value | 52 4 | 29 4 | 2 8 | 94 6 |
| Normal value | – | 4 9±2 1 | 5 6±2 1 | 10 5±2 9 |
| Lambda chains | | | | |
| Patient s value | – | 2 3 | 0 4 | 2 7 |
| Normal value | – | 5 2±0 5 | 2 7±0 9 | 7 9±1 1 |

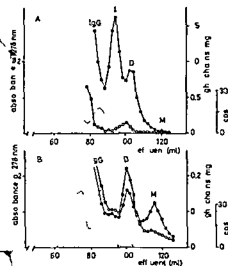


*Fig 1* Gel filtration on Sephadex G 100 columns (140× 1 5 cm) of 0 5 ml serum from the patient (*A*) and 0 5 ml normal serum (*B*) Distribution of protein in the fractions was determined by measuring the absorbance at 278 nm ( ) =the elution of I 125 labelled lambda chain dimers (cps) eluted at an effluent of 100 ml Kappa (●—●) and lambda (O—O) chains were determined by radioimmunoassay IgG represents regular immunoglobulins determined by radioimmunoassay *D*=dimeric *M* monomeric forms of lambda and kappa chains The arrow indicates the elution of the abnormal protein *Note* different scales for the light chain concentrations in Fig 1*A* and 1*B*



*Fig 2* Two glomeruli from autopsy specimen both showing mesangial nodules Arrow at normal afferent arteriole PAS staining ×150

creased Fig 1*A* shows the elution diagram obtained on gel filtration of serum from the patient and Fig 1*B* a normal serum filtrated on the same column A remarkably high peak of abnormal protein with kappa chain determinants in the patient's serum is eluted between albumin and dimeric forms of light chains The calculated molecular weight of this protein is about 55000 Furthermore Fig 1*A* shows that the fraction of monomers is very small Half kappa chains or lower molecular weight fragments of kappa chains were not found The elution diagram for lambda chains has a normal pattern the concentration however is diminished compared with normal serum Rechromatography of the abnormal peak revealed no evidence of dissociation of the protein

The 24-hour urinary excretion of kappa chains was 58 2 mg (normal range 1 4–5 7) that is about 10 times higher than the upper normal level The 24-hour urinary excretion of lambda chains was 4 6 mg (normal range 0 6–1 9) Immune complexes were not demonstrated by the two methods employed

### Pathology

*Renal biopsy two months before death* About one third of the 30 glomeruli seen on light microscopic examination were completely hyalinized Moderate to marked diffuse PAS positive mesangial widening was present in all of the remaining glomeruli and one fourth contained PAS positive lesions of nodular types as demonstrated in autopsy specimen (Fig 2) Each nodule was surrounded by a rim of capillaries The widened as well as the nodular mesangial regions were either homogeneous or finely granular All staining reactions for amyloid and fibrin were negative The peripheral capillary walls often appeared slightly thickened but were normal in some areas There was no hypercellularity or glomerular necrosis

Immunofluorescence microscopy with antibody against the kappa type of light chains demonstrated




*Fig 3* Pronounced nodular mesangial deposits of basement membrane like material in glomerulus from biopsy specimen An irregular electron lucent rim (*) is noted below the endothelial cells of adjacent capillary *CL* capillary lumen *Bo* =Bowmann s capsule *US* urinary space (×3 400)

deposition of kappa chains in the nodules There was no fluorescence with antibodies against lambda chains or heavy chains of IgG IgA IgM IgD or IgE The arterioles showed severe hypertensive changes partly with proliferative intimal thickening and arteriolonecrosis partly as muscular hyperplasia and hyalinosis A marked fibrous intimal thickening was noted in the interlobular arteries There was a rather pronounced diffuse tubular atrophy and interstitial fibrosis Some distal tubules contained dense homogeneous casts but typical changes of the myeloma type were not seen

*Electron microscopy* confirmed the presence of massive nodular mesangial expansions (Figs 3 4 5) consisting of basement membrane like material of finely granulated structure in places with a content of some very thin fibrils (<100 Å) without periodicity Irregularly arranged fibres 200–250 Å in width with a periodicity of about 150 Å were also present (Fig 5 inset) but neither fibrin nor amyloid fibrils were identified We did not observe so-called longspacing collagen as described by Schubert and Adam (18) The mesangial cells contained an abundant rough surfaced endoplasmic reticulum (Fig 4) often with dilated cisternae (some containing floccular material) well developed Golgi complexes and rather numerous mitochondria The peripheral basement membranes appeared to be of normal thickness in many places but were often difficult or impossible to measure due to an irregular electron lucent subendothelial rim in many capillary loops (Fig 3) A partial or complete detachment of the endothelial cells from the basement membrane was noted in places This gave rise to

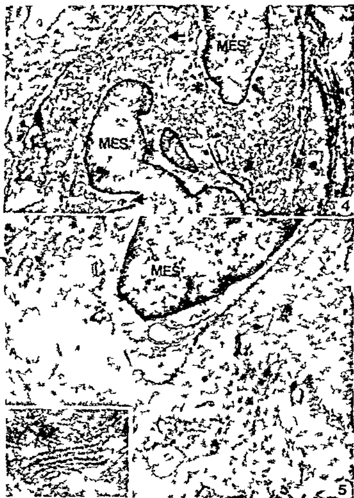



Fig 4 Detail of widened mesangial region showing mesangial cells (Mes) containing abundant rough surfaced endoplasmic reticulumum with dilated cisternae (arrows) including floccular material * Mesangial deposits (×7700)

Fig 5 Detail from mesangial nodule (×13 00) showing homogeneous or finely fibrillar deposits containing scattered coarser banded fibers some of which are shown in detail in the inset (×37400) Mes mesangial cell

thrombocyte aggregates and fibrin deposits in the capillary lumen and was attributed to the severe arterial hypertension

*Autopsy* disclosed slightly enlarged kidneys The glomerular changes had progressed practically all of the glomeruli showing several nodules (Fig 2) The arterioles were less severely affected and some of them showed no appreciable change (Fig 2)

The lymph nodes liver and spleen were normal also on histological examination Osteolytic lesions were not found on X ray examination The bones of the skull were thin and translucent Bone marrow histology was diagnostic of multiple myeloma showing a diffuse increase in the amount of plasma cells with immature and multinucleated forms

*Bone marrow* The immunoperoxidase technique showed that kappa chains were accumulated in the plasma cells Lambda type light chains or heavy chains of gamma alpha and mu type were not demonstrated

## DISCUSSION

The present case was classified as multiple myeloma because of a severe infiltration of mature and immature plasma cells in the bone marrow As the electrophoretic investigations of serum and urine showed decreased concentrations of all classes of immunoglobulins without any evidence of monoclonal proteins the case was designated as nonsecretory (9) Only by a sensitive radioimmunoassay was an increased concentration of kappa chains found in serum and urine The selective increased concen

tration of kappa chains associated with accumulation within the plasma cells of kappa chains only confirmed the monoclonal nature of the plasma cell proliferation

In approximately 1% of patients with multiple myeloma a monoclonal component cannot be demonstrated in serum and urine (9 15) Several cases of these nonsecretory myelomas have been described (1 2 7 11 16 27) Ultrastructural and immunohistologic investigations have indicated that multiple myeloma without an M component can be separated into nonproducers and true nonsecretors of immunoglobulins (11) The latter type is characterized by accumulation of monoclonal immunoglobulin within the plasma cells A selectively increased concentration of kappa chains was found in our patient The concentration in serum was 94 6 mg/l that is 5 fold the upper normal level and the urinary excretion was 58 2 mg/24 h This demonstrates that at least kappa chains are secreted from the abnormal plasma cells The term lowsecretory myeloma has also been ap- to these cases of myeloma characterized by [illegible] amount (≤0 1 g/l) of monoclonal components in serum and urine (27) It is suggested that no sharp limit exist between nonsecretory and lowsecretory myeloma (27) Demonstration of monoclonal components in these cases depends probably on the methods employed The M-component in our patient was of kappa type This agrees well with the preponderance of this light chain type in low and nonsecretory myelomas (27)

Heavy chains of gamma alpha and mu type were not demonstrated in the plasma cells by the immunoperoxidase technique This was interpreted as the plasma cells were nonproducers of heavy chains The concentrations of the normal immunoglobulins were severely depressed This agrees with findings by others in nonsecretory multiple myeloma (2 27)

Renal abnormalities are common in multiple myeloma. In about 55% of patients elevated serum creatinine is an initial finding and in about 15% the cause of death is uremia (9) The characteristic tubular lesions of myeloma type are found in most of these cases but cases of glomerular lesions resembling the nodular glomerulosclerosis of diabetes mellitus have been described recently (5) As in our patient the clinical course of these patients has been a rapidly progressing renal insufficiency terminating in uremia A case of nonsecretory myeloma with this characteristic glomerular lesion has also been described earlier (1) In this patient a severe hypogammaglobulinemia was found without any evidence of monoclonal components The plasma cell infiltrates showed fluorescence with both antigamma chain and antikappa antibodies Immunofluorescence studies of renal tissue were not performed The methods used by this group were not sensitive enough to detect free kappa chains in serum in the concentrations observed in our patient

The mechanism behind the formation of glomerular lesions in multiple myeloma is not clear Amyloid formation deposition of immune complexes immunoglobulins or immunoglobulin fragments have previously been suggested as possible pathogenetic factors (3 5 13) Two different methods showed no evidence of circulating immune complexes in our patient Immunofluorescence showed a deposition of kappa chains in the glomerular nodules a finding which has also been recorded in earlier studies (5, 12 16) Indeed a remarkably high frequency of monoclonal kappa type protein abnormalities has been observed in patients with multiple myeloma and nodular glomerulosclerosis In one case only has another type of M component been demonstrated in association with this glomerular lesion (i e the Fc fragment of IgG (24)) It is well established that light chains of immunoglobulins give rise to the fibril proteins of primary amyloidosis but these fibrils are usually derived from the lambda type light chains (6) In addition there was no evidence of amyloid deposits on light microscopy Although electron microscopy showed fine fibrils in the deposits they were not typical of amyloid and their nature is uncertain They resemble the fine fibrils described by Thiele et al (26) in early renal lesions of paraproteinemic patients and suggested by these authors to be precursors of amyloid fibrils The coarse fibers with a periodicity of about 150 Å are similar to those described by Dachs et al (4) in diabetic nodular glomerulosclerosis and considered to be precollagen An alternative possibility is that the fibers represent paraprotein aggregates as proposed by Thiele et al (26) who found similar banded fibers

The theory has been proposed that mesangial accumulation of paraprotein may induce an increased synthesis (or reduced catabolism) of basement membrane (13) leading to the formation of nodules This theory gains support by our findings

since there was evidence of both paraprotein and basement membrane deposition Kappa chains were demonstrated in the lesions which contained basement membrane like material and mesangial cells with signs of increased synthetic activity were seen

Polymeric forms of free light chains in serum from patients with nodular glomerulosclerosis and multiple myeloma have not been investigated be fore In our investigation about 2/3 of the kappa chains were eluted from the Sephadex G 100 col umns corresponding to an abnormal molecular weight of 55 000 and 1/3 with a molecular weight of 44 000 corresponding to dimeric forms of kappa chains Only a minor fraction was eluted corre sponding to monomers with a molecular weight of 22 000 Rechromatography of the abnormal protein eluted with a molecular weight of 55 000 showed no evidence of spontaneous dissociation In normal serum one half of the kappa chains exist as partly dissociable dimers one half as monomers and a very small fraction as tetramers (22 23) Thus a highly abnormal tendency to formation of stable polymers of kappa chains was found in our patient Recently we have also demonstrated an increased tendency for light chain polymerization especially of lambda type in serum from patients with pri mary localized amyloidosis (19) The abnormal kappa chain protein may represent a half kappa chain combined with dimers or it could be kappa chains in association with unknown protein Free half kappa chains were not present The signifi cance of the formation of a stable abnormal poly merization product of kappa chains is not clear but perhaps the abnormal kappa chain protein accumu lated in the renal mesangial cells has provoked the formation of the glomerular nodules

## ACKNOWLEDGEMENT

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## REFERENCES

1 Arend W P & Adamson J W Nonsecretory myeloma Immunofluorescent demonstration of para protein within bone marrow plasma cells Cancer 33 721 1974
2 Azar H A Zaino E C Phan T D & Yannopoulos K Nonsecretory plasma cell myeloma Observation on seven cases with electron microscopic studies Am J Clin Pathol 58. 618 1972
3 Beaufils M & Morel-Maroger L Pathogenesis of renal disease in monoclonal gammopathies. Current concepts Nephron 20 125 1978
4 Dachs S Churg J Mautner W & Grishman E Diabetic nephropathy Am J Pathol 44 155 1964
5 Ganeval D Jungers P Noel L H & Droz D La nephropathie du myelome In Actualites nephrologiques de l'Hospital Necker pp 309-344 Flammarion Paris 1977
6 Glenner G G & Page D L Amyloid amyloidosis and amyloidogenesis Int Rev Exp Pathol 15 1 1976
7 Huez D Preud Homme J L & Seligman M Intracellular monoclonal immunoglobulin in nonsecretory human myeloma. J Immunol 104 263 1970
8 Indiveri F Barabino A Santolini M E & Santolini B "Nonsecretory multiple myeloma Report of a case Acta Haematol 51 302 1974
9 Kyle R A Multiple myeloma. Review of 869 cases Mayo Clin Proc 50 29 1975
10 Laurell C B Quantitative estimation of proteins by electrophoresis in agarosegel containing antibodies Anal Biochem 14 45 1966
11 Mancilla R & Davis G L Nonsecretory multiple myeloma Immunologic and ultrastructural observations on two patients Am J Med 63 1015 1977
12 Noel L H Droz D Ganeval D & Jungers P Glomerulosclerose nodulaire du myeloma. Un depot exclusive de chaines legeres dans le mesangium Abstract. VIIth International Congress of Nephrology Montreal Canada 1978
13 Olsen T S Mesangial thickening and nodular glomerular sclerosis in diabetes mellitus and other diseases Acta Pathol Microbiol Scand (A) (Suppl) 233 203 1972
14 Olsen T S Posborg Petersen V & Sommer Hansen E Immunofluorescence studies of extracapillary glomerulonephritis Acta Pathol Microbiol Scand (A) (Suppl) 249 20 1974
15 Osserman E F & Takatsuki K Plasma cell myeloma. gammaglobulin synthesis and structure A review of biochemical and clinical data, with the description of a newly recognized and related syndrome Hy -chain (Franklins) disease Medicine 42 357 1963
16 Randall R E Williamson W C Jr Mullinax F Tung M Y & Still W J S Manifestations of systemic light chain deposition Am J Med 60 283 1976
17 River G L Tewksbury D A & Fudenberg H H Nonsecretory" multiple myeloma. Blood 40 204 1972
18 Schubert G E & Adam A Glomerular nodules and long spacing collagen in kidneys of patients with multiple myeloma J Clin Pathol 27 800 1974
19 Sølling J & Sølling K Free light chains of immunoglobulins in amyloidosis Acta Med Scand 206 283 1979
20 Sølling J Sølling K & Jakobsen K U Circulating immune complexes in lupus erythematosus scleroderma and dermatomyositis Acta Derm Venereol In press 1979
21 Sølling J Sølling K Jakobsen K U & From E

Circulating immunocomplexes in syphilis Acta Derm Venereol 58 263 1978
22 Solling K Free light chains of immunoglobulins in normal serum and urine determined by radioimmunoassay Scand J Clin Lab Invest 35 407 1975
23 — Polymeric forms of free light chains in serum from normal individuals and from patients with renal diseases Scand J Clin Lab Invest 36 447 1976
24 Solling K & Askjær Sv Aa Multiple myeloma with urinary excretion of heavy chain components of IgG and nodular glomerulosclerosis Acta Med Scand 194 23 1973
25 Taylor C R & Mason D Y The immunohistological detection of intracellular immunoglobulin in formalin paraffin sections using the immunoperoxidase technique Clin Exp Immunol 18 417 1974
26 Thiele K Kuhn K Zobl H & Krull P Die fruhen morphologischen Veranderungen der menschlichen Niere bei Paraproteinamie Eine elektronmikroskopische Untersuchung Beitr Pathol 157 349 1976
27 Turesson I & Grubb A Non secretory or low secretory myeloma with intracellular kappa chains Acta Med Scand 204 445 1978

## ANNOUNCEMENTS

*First World Biomaterials Congress* will be held in Baden Austria, near Vienna April 8–12 1980 The official language will be English

The achievements outstanding problems and future trends in the following areas will be reviewed Materials behaviour Tissue behaviour Blood behaviour Dressings Suture and tissue adhesives Plastic and reconstructive surgery Dental and maxillofacial Ophthalmology Orthopaedics Cardiovascular surgery Artificial organs Drug delivery systems Regulation of biomaterials devices

*Information* World Biomaterials Congress Secretariat Ms E Maurer Medical Academy of Vienna Alster Strasse 4 A 1090 Vienna Austria

*The International Society and Federation of Cardiology (ISFC)* has an intensive programme of scientific and public educational activities These activities include the work of its Public Education Committee and of its Scientific Councils on Arteriosclerosis Cardiomyopathies Epidemiology and Prevention Hypertension Paediatric Cardiology Rehabilitation of Cardiac Patients Thrombosis and Haemostasis A new Scientific Council on Cardiac Metabolism is being formed Members of these Councils act as advisers to the Public Education Committee

The Scientific Councils organize multinational research studies teaching seminars and postgraduate courses on such subjects as congestive cardiomyopathies endomyocardial fibrosis mild hypertension epidemiology and prevention of cardiovascular disease Task forces are being formed to examine the current position of thrombosis and drugs which inhibit platelet function and other urgent issues Teaching and research fellowships are planned and an annual international lectureship is in prospect

In collaboration with the World Health Organization task forces have been working on proposed international classifications and definitions of arrhythmias ischaemic heart disease cardiomyopathies and haemodynamics The first two task forces have already issued their reports [1] while those on cardiomyopathies and haemodynamics are under preparation

A manual on the control of hypertension for general practitioners is being prepared also in collaboration with WHO

Close liaison is maintained with WHO in the field of public education and prevention Workshops and conferences have been held in 1979 and are being arranged in 1980 to emphasize the cardiovascular dangers of cigarette smoking

The ISFC sponsors World Congresses of Cardiology which are held every four years and participates in the planning of the scientific programme

Further information may be obtained from the ISFC Geneva Office P O Box 117 1211 Geneva 12 Switzerland

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[1] Definition of terms related to cardiac rhythm Report of a WHO/ISFC Task Force Am Heart J 95 796 1978 Eur J Cardiol 8/2 127 1978 and Arch Mal Cœur (Suppl) 6 1979 Part II Classification of cardiac arrhythmias and conduction disturbances Am Heart J 98 263 1979

Nomenclature and criteria for diagnosis of ischemic heart disease Report of the Joint ISFC/WHO Task Force on Standardization of Clinical Nomenclature Circulation 3 607 1979 French version will be published in Arch Mal Cœur

Acta Med Scand 207: 145–149, 1980

DEBATE

# Diet, Lipids and Atherosclerosis

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ABSTRACT. During the Second World War there was a temporary reduction in the frequency of myocardial infarction in Finland, Norway and Sweden. This was probably due to the reduced consumption of saturated fat. The total amount of dietary fat and cholesterol presumably played only a minor role. Today it is easier to obtain reliable mortality figures and information about the fat composition of the food in a given country. A compilation of such data has shown that mortality from myocardial infarction is higher in countries with a high consumption of saturated fat. Polyunsaturated fats seem to have an opposite effect. There is no reason to dissuade people from using fat-modified food products with a lower fat content and replacement of part of the saturated fat by polyunsaturated. Refugees from countries where the diet contains very little fat should be warned against using too much of the high-fat food products widely consumed in their new countries.

*Key words:* diet, lipids, atherosclerosis, Second World War.

Acta Med Scand 207: 145, 1980

In an article in a recent number of the Acta Medica Scandinavica (19) entitled "Diet, lipids and heart attacks", Lars Werkö took up the question whether the composition of the diet during the Second World War had had any effect on the mortality from atherosclerotic heart diseases at that time. He does not believe that it had, and he adopted a very critical attitude to a statistical investigation of the problems published by me (10).

## MORTALITY STATISTICS

Werkö seems to have forgotten that my article was published 30 years ago when the whole situation and the statistical material available for medical research were quite different from what they are today. As far as atherosclerotic and degenerative heart diseases are concerned, it was not until 19[illegible] that the official publication "Causes of deaths" gave the number of deaths per 100 000 inhabitants among men and women in different age groups. Neither were the deaths assigned to different groups according *to exactly the same principles in all the countries*. The low mortality rate in Norway can probably be explained in part by the fact that arteriosclerosis was not registered as a cause of death if a more definite diagnosis was also given in the death certificate. On inquiry at the official statistical centres in the Nordic countries and the USA, I was informed that in the years in question, 1935–47, the principles of assignment of the cases to the various groups had not been changed. Though *it is not possible to compare the mortality at that time in one* country with that in another—as Werkö has done now—a change in the mortality curve in each particular country might be of interest.

In 1949 I sent a questionnaire to the chief physicians of about 100 hospitals in the countries in question requesting figures on the frequency of patients with the diagnosis of myocardial infarction relative to the total number of patients admitted to the department and the number of deaths in 1939–48. In *this way I collected a material consisting of about* 7500 cases with this diagnosis from [illegible]3 departments of internal medicine in Sweden, Norway and Denmark (9). Judging from these figures, the number of deaths decreased somewhat in Norway and Sweden during the war and the total number of patients admitted to hospital because of myocardial infarction increased substantially after the war. No such variation was found in Denmark.

Some years later, in an effort to check the reliability of the diagnosis of coronary thrombosis given in the death certificates in Sweden, Siv Borgström and I studied in cooperation with the Swedish

Statistical Central Office 3585 death certificates (12) By personal letters to the physicians concerned we obtained information about the findings on which the diagnosis had been based in every single case In our opinion the diagnosis was fairly certain in 77% and probable in a further 9% The diagnosis had been confirmed at necropsy in 26% of the cases 40% had been hospitalized

The cause of death is of course most certain if confirmed at autopsy Folke Henschen published a compilation of deaths from arteriosclerosis + chronic myocardial inflammation confirmed at autopsy at the institutes of pathology in Stockholm in 1928–45 (5) and found that the frequency of these diagnoses clearly declined in 1942 and 1943 Vartiainen and Kanerva reported a similar investigation of cases from the Institute of Pathology in Helsinki in 1933–38 and 1940–46 (17) Their investigation revealed that the atherosclerotic changes found at autopsy were much smaller during the latter period when Finland was at war and food—high fat products in particular—was scarce

In a very recent publication on cardiovascular diseases during World War II (13) Gotthard Schettler gave an interesting description of the situation in the Federal Republic of Germany and the frequency of myocardial infarction during the final years of the war and the early postwar period No reliable data on the mortality from myocardial infarction at that time is available In the departments of pathology in Tubingen Marburg and Stuttgart however no cases of myocardial infarction were seen in 1945–48 At that time the Germans were living on a very lean diet After 1948 the food situation improved quickly and as early as in 1950 the frequency of deaths from cardiovascular disease began to rise substantially This rise continued until 1970 and did not seem to stop until 1974

Another interesting observation during the lean postwar years was a fall in the frequency of thromboembolism In Norway Strom and Adelsten Jensen (14) found a definite fall in the frequency of postoperative thrombosis and embolism during the war They also found that the mortality from arteriosclerotic heart disease was lower during the war in all age groups particularly in the younger After the war the mortality rate rose in all age groups above 40 years

All these observations in the various countries point in the same direction and it is beyond all doubt that the frequency of myocardial infarction fell temporarily during the Second World War in certain countries Certain observations also indicate that the frequency of postoperative thrombosis and embolism fell during the same period A question that unsought arises is What was the cause of this temporary fall? As before (18) Werko feels that the decrease in arteriosclerotic deaths was due to sulfa drugs and the wartime lack of respiratory epidemics after an influenza epidemic in the late 30s I find it difficult to understand how sulfa therapy of infections should lower the mortality from myocardial infarction and if it did why did the decreases last for only 2–3 years According to Lofstrom (8) since the large influenza epidemics in 1918–20 and 1922 we in Sweden have had only relatively mild epidemics of influenza though the sick rate was perhaps somewhat higher in 1927, 1931 and 1939 But we also had such epidemics during the war with rather high morbidity in 1941 It thus hardly seems likely that the variation in mortality from myocardial infarction can be ascribed to the presence or absence of influenza epidemics

## CONSUMPTION OF FAT DURING THE WAR

When I wrote the above mentioned article (10) 30 years ago I knew but little about the cause of arteriosclerotic disease With knowledge of Anitschkow s animal experiments (1) and Block and Rittenberg s synthesis of cholesterol from fat via acetate (3) it struck me that there might be some connection between myocardial infarction atherosclerosis cholesterol and high fat food products I studied the mortality from myocardial infarction and the consumption of fat particularly animal high-cholesterol food products It was however not known at that time that saturated fat differs from polyunsaturated fat in its effect on the blood cholesterol level This difference was discovered later by Kinsell et al (7) and Groen et al (4) It is therefore quite possible that under such circumstances my calculations were misleading It might however not be out of place once again to have a brief look at the changes in the food supplies in the various countries during the war

In Finland the consumption of milk and butter decreased during the wars Also the manufacturing of margarine was reduced and in 1943 and 1944 it was completely stopped At that time margarine

was produced mainly from copra and animal fats both of which are saturated It was not until 1945 that Finland began to grow oleiferous plants Consequently mainly saturated fat was used in Finland during the wars though in a substantially smaller amount than in peace time Consumption of milk fat increased after the wars and was doubled by 1963

Denmark an agricultural country was occupied during the war but the production of food continued more or less undisturbed However it was not possible to import copra whale fat fish oil or soy beans previously used in the manufacturing of margarine The production of margarine decreased rapidly and was discontinued for 4 years The loss of margarine was substituted by butter much of which had been exported to England before the war and the consumption of butter doubled Neither was meat scarce and any serious shortage of food never existed Thus the consumption of saturated fat remained high during the war as did the mortality from myocardial infarction

Neither in the USA did the war have any noteworthy effect on the supply of food produc s the high consumption of saturated fat continued And no reduction of the mortality from myocardial infarction was recorded It was not until some 15–20 years ago that the Americans began to use liquid vegetable oils to a greater extent with increasing consumption of polyunsaturated fat as a result Judging from available figures this has now had a favourable effect on the mortality from atherosclerosis in the USA It should however be pointed out that the total fat consumption in the USA is still high and the ordinary diet still contains very much saturated fat

Before the war Sweden imported fairly large quantities of copra and whale fat which were used mainly in the margarine industry The whale oil was hardened before use During the war this import decreased substantially The import of copra decreased from 30 000 tons a year to nil but increased afterwards to 10 000 tons a year and remained at this level for the following 5 years Sweden started to grow oleiferous plants mainly rape but also mustard and poppies as a substitute for the earlier imported fat The liquid vegetable oils obtained were used in their natural state i e they were not hardened From 1940 to the spring of 1949 edible fat was rationed Though the ration was fairly generous the total consumption of fat decreased Moreover the fats used probably contained a larger proportion of polyunsaturated fatty acids at the expense of saturated fatty acids

The food situation in Norway during the war undoubtedly scarcer than in Sweden has been studied and reported bv Strom and Adelsten Jensen (14 15) They reported that the supply of calories especially fat-containing foods was reduced The average calorie content of the food fell from about 3 500 to 2 800 calories and resulted in a widespread loss of weight People used less butter margarine whole milk and meat but more skim milk cereals potatoes fresh vegetables and much more fish Moreover many people took a tablespoon of cod liver oil a day It may therefore be assumed that the consumption of fat especially saturated fat was much lower and that the diet contained relatively more polyunsaturated and less saturated fat during the war than before

This review of the investigations during the Second World War thus suggested the possibility of a certain connection in Finland Norway and Sweden between the temporary fall in mortality from myocardial infarction and the decreased consumption of saturated fat and proportionally somewhat increased use of polyunsaturated fat It was this possibility that aroused my interest in the cause of atherosclerosis and the possibilities of preventing it As known this is the disease that for several years has received the widest attention in modern medicine and literature In his recent article (19) Werko is of course quite right when he says that sudden death from a heart attack is due to special factors which have nothing directly to do with the patient s earlier diet and blood lipid level But it cannot be denied that there are practically always atherosclerotic changes in the coronaries i e changes which may have something to do with heart attacks The most important thing is therefore to prevent the development of atherosclerosis and here the question of the fat composition of the food products comes into the picture

## DIETARY TRIALS

In the last two or three decades several dietary trials have been run to find out whether it is possible to prevent the disease As the end point of such trials the investigators have nearly always used myocardial infarction fatal and non fatal plus sudden coronary death Some of these trials have given

positive results others negative or uncertain The difference can probably be explained by differences in the design of the trial and in the extent to which the participants observed the diet Broadly speaking such participants were nearly always patients i e persons who had already had an infarction or adults who admittedly felt well but who were old enough to have developed atherosclerotic changes in certain vascular areas Many of the participants had high total cholesterol values already at the beginning of the trials The formerly accepted so called normal cholesterol level was far too high The real optimal level of the total cholesterol is about 150 or at any rate below 200 mg/100 ml

*A dietary trial able to produce a final proof* and give a definite answer to the question whether atherosclerosis can be prevented by a suitable diet should be started on young persons with an initially optimal cholesterol level In addition the trial should be continued for several years during which the participants should observe the dietary restrictions But such a trial would never be possible in practice

Is there any other possibility of testing whether the choice of suitable food products can have a prophylactic effect or a therapeutic effect on existing atherosclerosis? One method to check the possibility of a therapeutic effect has now become available (2) i e visualization with the aid of computer technology of atherosclerotic plaques in the femoral artery It is now also easier at least in Sweden to alter the diet thanks to the fat modified food products on sale in ordinary food stores It has been clearly shown in the so-called Klostergård trial (11) that lowering of the blood cholesterol in a normal population is possible with the aid of such natural products which contain a smaller amount of saturated fat and in some of which a part of the saturated fat has been replaced by unsaturated That trial carried out on 650 persons belonging to the normal population in a residential area was not a conventional long term trial in which every case is followed up for several years to find out whether the diet has any prophylactic effect on atherosclerosis The purpose was instead to ascertain whether the suggested diet with the fat modified food lowered the blood cholesterol level and secondly whether it was easy to persuade people in general to change their dietary habits After having been informed of the nature of the intended trial it was quite easy to get the residents to try the new

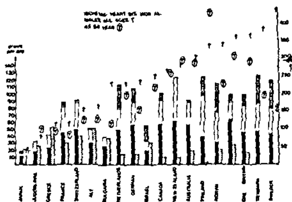


*Fig 1* Fat available and mortality from ischemic heart disease among men in 19 countries ■=Meat fat □=milk fat ⊟=margarine ⊠=vegetable oil (From World Health Statistics Annual vol 1 Causes of Death and F A O Provisional Food Balance Sheets 1972–74 )

fat modified food products and to continue to use them The blood cholesterol fell in practically all participants in some of them as much as 75–100 mg/100 ml

## FAT MODIFIED FOOD FOR ALL

The next question is whether it is justified on the basis of such a simple trial to inform the Swedish population of this possibility of influencing the blood cholesterol in the right direction i e lowering it to an optiamal level of about 200 mg/100 ml or still somewhat lower If one scans the literature strong argumentsfor such a policy will soon be found Thus it is obvious from Fig 1 that the mortality from ischemic heart disease is much higher in countries with high than low consumption of especially saturated fat The use of polyunsaturated fat in the form of vegetable oil seems also to have a desirable effect Other factors such as smoking naturally play a role but only as contributory causes of the high mortality from cardiovascular diseases Thus it is known that the consumption of cigarettes in Japan is very high yet the mortality from myocardial infarction is somewhat lower than in any of the 19 countries in this study It is also well known that Japanese who have migrated to the USA often develop higher cholesterol values and after a time also myocardial infarction

Such a series of events the opposite to what happened during the Second World War is well worth bearing in mind Large population groups

from South East Asia are moving today to countries with much higher standard of living and consumption of saturated fat These unfortunate people should be served a nutritious diet with relatively little fat—preferably with due proportion of polyunsaturated fat During the transitional period i e until they prepare their meals themselves they should be warned of the risks of adopting the high fat food of the country to which they have migrated Food containing relatively little fat polyunsaturated rather than saturated can be useful for all of us

When studying the effect of various fats in dietary trials or in various ethnic groups it might be of interest not only to determine the total cholesterol value but also the values of the various lipoprotein fractions Of special relevance in this connection are the low density lipoproteins (LDL) and the high density lipoproteins (HDL) A simple method elaborated by Swahn (16) and based on paper electrophoresis was used already in 1956 in an investigation comparing the lipoprotein patterns and dietary habits between Italians and Swedes (6) If the diet is changed and supplied with more saturated fat LDL rises and, consequently also the total cholesterol value The HDL fraction is of particular interest as regards individual risk assessment in high risk affluent cultures

When attempts are made to inform the public about these interesting and rather complicated problems the expression the good cholesterol is often used instead of HDL The public unfortunately has often misunderstood this denomination They have accordingly been led to believe that all cholesterol is good and useful and in any case not so bad that all what is necessary is to eat a couple of eggs every day and to jog a little in the forest so that the useful cholesterol rises this the experts have said! Can we rely on these statements? What have the HDL-experts to say about this?

## REFFRENCES


1 Anitschkow N Beitr Path Anat 56 379 1913
2 Blankenhorn D H Brooks S Selzer D H & Barndt R Circulation 57 355 1978
3 Block K & Rittenberg D J J Biol Chem 143 297 1942
4 Groen J Tjiong B K Kamminga C E & Willebrandt A F Voeding 13 556 1952
5 Henschen F Verdandis Småskrift 491 1947
6 Keys A Andersson J T Aresu M et al Clin Invest 35 11 1956
7 Kinsell L W Partridge J Bolin L Margen S & Michaels G J Clin Endocrinol 12 909 1952
8 Lofstrom G Acta Med Scand 133 253 1949
9 Malmros H Nord Med 42 1785 1949
10 — Acta Med Scand (Suppl) 246 137 1950
11 — Ann NY Acad Sci 300 379 1977
12 Malmros H & Borgstrom S Z Kreislaufforsch 49 201 1960
13 Schettler G Prev Med 8 581 1979
14 Strom A & Adelsten Jensen R Nord Hygien Tidskr 31 123 1950
15 — Lancet 1 126 1951
16 Swahn B Scand J Clin Lab Invest 4 98 1952
17 Vartiainen I & Kanerva K Ann Med Intern Fenn 36 748 1947
18 Werko L Lakartidningen 64 3598 1967
19 — Acta Med Scand 206 435 1979

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# LETTER TO THE EDITOR

*Dear Sir*

Lars Werko s important critique of the lipid–heart theory (15) is timely and excellent I have already published many arguments against the widespread credulity that polyunsaturated fat (PUF) in the diet can exert any protective or preventive effect on the incidence of coronary heart disease (8–12)

The recently completed clofibrate primary prevention trial (5) has given little encouragement to efforts to reduce blood cholesterol Reduction of non fatal infarcts is only marginally significant but if we take into account the whole picture of new coronary events the results scarcely reach statistical significance

The principal protagonists of PUF diets began with the aim of reducing blood cholesterol which can be achieved The increased diversion of cholesterol from the blood through the liver by PUF diets was predicted by Borgstrom (4) to increase *biliary stone troubles and this prophecy has been* borne out by the clofibrate trial Kannel and Castelli (7) admit disappointment with the results of cholesterol lowering trials but now want to consider HDL/LDL ratios There is however no evidence that this ratio will be favourably influenced by present PUF dietetic recommendations Another shift in their advocacy is that the diet will supply substrate for prostaglandins (2) but even this speculation is discouraged by recent experimental work which shows that sunflower seed oil depresses the formation of PGI which is the product inhibiting platelet agglutination and possibly thrombosis Thus the belated search for other justification of their dietetic recommendations finds no scientific basis

The confusion of thought now besetting the proponents *who wish to change our diet is exemplified* in the National Food Policy publication (13) pp 86–88 These protagonists finally claim that numerous international committees who in the past have recommended PUF diets together with the 92% support for Norum s dietetic enquiry constitute good enough evidence This is quite astonishing No scientific problem has ever been resolved by vote and Norum s enquiry was obviously directed to doctors who were already hoping for some improvement by putting themselves on the diet This is hardly a fair sample as Werko points out

A careful study by Beaglehole et al (3) on a population of New Zealand Maoris extended over a decade shows that those with the lowest blood cholesterol had the highest mortality from all causes at all ages The authors call attention to a similar observation in the Framingham studies So it is possible that this hazard may not be restricted to Polynesians The work of Vesselinovitch et al (14) and Wissler et al (16) on primates indicates that certain vegetable oils can be more damaging to blood vessels than butter particularly coconut oil and peanut oil

It is thus clear that attempts to keep cholesterol low by changing to margarines containing vegetable oil has been shown to be of little value and indeed could be damaging in certain instances I go a little further than Werko who accepts that in the highest *levels of the normal cholesterol range (<300 mg)* there can be an association with later development of coronary heart disease My personal view is that it is not necessary to attempt to reduce cholesterol in the normal range unless one is dealing with a rare familial abnormality in the heterozygotes of familial hypercholesterolaemia This is the only condition in which high cholesterol is likely to contribute to the progress of ischaemic heart disease

Ahrens (1) who has worked on this subject for three decades now comes to the conclusion that it is irresponsible to press PUF recommendations on the general public His long experience and careful scientific studies suggest that we should as a profession cease to promote the commercial interests which by aggressive advertisement try to win *profits for themselves It will certainly profit no one* else

Yours

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## REFERENCES

1 Ahrens E H Dietary fats and ischaemic heart disease Lancet 2 1345 19 9
2 Ball K Prevention of coronary heart-disease Lancet 2 118_ 1979
3 Beaglehole R Foulkes M A Prior I A M &

Eyles E F Cholesterol and mortality in New Zealand Maoris Br Med J 1 285 1980
4 Borgstrom B Livsmedelteknik 7 302 1976
5 Committee of Principal Investigators A co operative trial in the primary prevention of ischaemic heart disease using clofibrate Br Heart J 40 1069 1978
6 de Deckere E A M Nugteren D H & ten Hoor F Influence of types of dietary fat on the prostaglandin release from isolated rabbit and rat hearts and from rat aortas Prostaglandins 17 947 1979
7 Kannel W B & Castelli W P Is the serum total cholesterol an anachronism? Lancet 2 950 1979
8 McMichael J Dietetic factors in coronary disease Eur J Cardiol 5 447 1977
9 — Fats and atheroma an inquest Br Med J 1 173 1979
10 — Fats and arterial disease Am J Cardiol 98 409 1979
11 — Fats and atheroma Br Med J 1 890 1979
12 — Why blame cholesterol? Lancet 2 1182 1979
13 National Food Policy U K University of Reading 1979
14 Vesselinovitch D Getz G S Hughes R H & Wissler R W Atherosclerosis in the rhesus monkey fed three food fats Atherosclerosis 20 303 1974
15 Werko L Diets lipids and heart attacks Acta Med Scand 206 435 1979
16 Wissler R W Frazier L E Hughes R H & Rasmussen R A Atherogenesis in the Cebus monkey Arch Pathol 74 312 1962

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# Coronary Heart Disease, Serum Cholesterol and the Diet

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*The scientific method demands that every scientific* hypothesis be periodically re examined in the light of relevant new facts and new analysis of the evidence In principle then we should welcome updating and re evaluating of the hypothesis which Werko calls the diet–heart theory or lipid–heart disease theory (31) Here as elsewhere Werko is imprecise All English dictionaries agree with the Oxford Dictionary definition going back to 1638 In science a theory is a hypothesis that has been confirmed or established by observation or experiment and is propounded or accepted as accounting for the known facts The present question does not warrant the term theory we are concerned with the hypothesis that there is a relation between the development of coronary heart disease the concentration of lipids particularly cholesterol in the blood plasma and the habitual diet

Unhappily some re examinations of this hypothesis are patently expressions of bias even *commercially inspired ex parte statements like* those sometimes heard in courts of law when a contending lawyer presents only such bits of evidence as seem to support his side and uses all devices to *ignore and discredit the contrary facts In this man*ner Werko has produced an ex parte argument that would be consigned to the trash basket were it not for the fact that the author is a prominent physician who could persuade or at least confuse colleagues not well informed in this field So he must be answered

Werko s introduction to discussion starts with a major misstatement The diet–heart theory or lipid–heart disease theory rests on certain correlations that have been accepted as valid facts such as the relation between atherosclerotic heart disease and food consumption during the Second World War in certain countries and in some controlled *intervention trials* But these are *not* the basis of the hypothesis they are only examples of findings offered as being consistent with the hypothesis—and they are by no means the most important Werko chooses to ignore the truly consequential evidence—the enormous literature on experiments on animals including monkeys and anthropoid apes the rigorously controlled dietary experiments showing the effect on the plasma lipids in man the large and growing literature on the epidemiology of coronary heart disease apart from Malmros report on the wartime experience that he attacks with such malice As a matter of fact Werko does not even explicitly state the theory he seeks to destroy

Werko ridicules the seminal 1950 review by Malmros because it made no mention of the rationing of gasoline and cigarettes and the fact that the crude mortality figures indicate a death rate from arteriosclerotic disease in Sweden higher than that in the United States or in Finland But is it possible that Werkö *is ignorant of the fact that the crude* death rate is greatly dependent upon the age structure of the population and that the population of Sweden was much older that that of the United *States and Finland? As to gasoline and cigarettes* Werkö must well know that Denmark was no more abundantly supplied than Norway and Sweden during the war but had no significant reduction of saturated fats and cholesterol in the diet—and unlike the other Scandinavian countries Denmark had no decline during the war in the death rate from arteriosclerosis

The risk of smoking cigarettes was only recognized long after Malmros wrote his 1950 paper so it is unfair to upbraid him for not considering that variable Moreover Werko gives no figures to support his suggestion that cigarette rationing during the war could account for a major decline in the

mortality from arteriosclerotic disease It is notable that until after the Second World War women in Scandinavia were rarely cigarette smokers yet their mortality from arteriosclerosis declined like that of the men Cigarettes were rationed in Great Britain too but the mortality from arteriosclerosis changed little Was that only coincidental to the fact that there was little change in the per capita fat consumption in that period?

Werko s implication that the use of gasoline is a risk factor suggests ignorance or at least disregard of the epidemiological method as I define it All conceivable variables are fair game in epidemiological exploration but it is not justifiable to propose that an observed association means cause and effect unless there are other reasons favoring causation If Werko seriously means that the use of gasoline promotes atherogenesis and myocardial infarction it is incumbent on him to indicate possible mechanisms I suspect however that this is just another example of an effort to confuse the issue by introducing random noise

Werko makes much of doubts about the accuracy of diagnoses in the 40s but not many students would agree with him that coronary heart disease was a disorder not yet clearly defined at that time or with his repeated insistence that coronary heart disease should not be inferred from arteriosclerotic heart disease on a death certificate This last is remindful of the remarkable freedom from coronary heart disease claimed for the Italo-American community of Rosetto because their death certificates read arteriosclerotic heart disease instead of myocardial infarction (7 8)

We admit that the diagnosis of coronary heart disease or arteriosclerotic heart disease was more commonly missed 30 years ago than now but that is not the crucial point If misdiagnosis were the explanation of the changes in the reported mortality in the 40s we should have to believe that in the Scandinavian countries except for Denmark the disease was increasingly misdiagnosed as the war went on but was again properly recognized during the post war years Moreover this remarkable explanation would have to insist that during those years there was no such change in diagnostic accuracy in the United States and Denmark! But there is much more information on the wartime experience of coronary heart disease than in the 1950 paper of Malmros so derided by Werko

Changes in atherosclerosis associated with dietary changes imposed by dietary restrictions in Germany in the aftermath of World War I were noted by Ludwig Aschoff (1) He published no numerical details but data showing striking decreases in the frequency of severe atherosclerosis in Finland associated with reduced dietary fat during World War II were published by Vartiainen and Kanerva (28 29) Either Werko is ignorant of these reports or he would find it too difficult to argue that the same pathologists changed their criteria coincidently with the changing diets of the populations

Malmros pioneer article was followed by a careful review of the experience in Norway where the German invasion and occupation had produced a progressive sharp decrease in animal fats in the diet (24) The decrease in arteriosclerosis mortality associated with the change in the diet was even more impressive than had been reported by Malmros and the resumption of foods high in saturated fats after the war was followed by a marked increase in the mortality Strom published more evidence in 1954 including the interesting fact that the same surgeon in Oslo found that the frequency of thromboembolic complications after specified operations changed strikingly in association with the dietary changes imposed by the war—a progressive sharp fall during the war and a rapid increase in the incidence with the resumption of eating diets relatively high in fats We are reminded of the experience of Dutch surgeons in Java with Javanese patients on their usual low fat diet Chinese on a diet higher in fats and Dutch patients adhering to the customary rich diet of the Netherlands (27)

In the Netherlands and in Western Germany too the death rate from coronary heart disease showed marked changes associated with the dietary situation in the war years and after (21 22) The time relations in the changes in the several countries are most significant In Norway and Finland where the shortage of food fats came early the decline in the coronary mortality started soon after in the Netherlands where the dietary change only began at the end of 1943 the change in mortality was evident only in 1944 and 1945 In Germany the main dietary change was late in the war and in the post war years and the change in mortality corresponded (4)

Populations changing diets as a result of migration rather than war are also of interest and a number of examples have been rather extensively studied notable the Yeminites migrating to Israel and Japanese migrating to Hawaii and then to con

tinental United States Space does not permit a review here especially since I have referred to much of the material over the years (5 6 9 10) In contrast with the reports of the experience in war time concerning the association of a decrease in coronary heart disease following a decrease in animal fats in the diet the migrants studied have generally changed from a low fat diet towards a much richer diet that of the Jews of European origin in Israel and the usual American diet In each case the first response noted to the change in the diet was an increase in the concentration of cholesterol in the blood followed by a more gradual rise in the incidence of coronary heart disease in later years

The findings of migrating populations are like those in populations changing their diets because of war they do not prove that the changes in the diet were directly responsible for the changes in the incidence of coronary heart disease other things besides the diet were changing But again the findings are consistent with the theory that the incidence of the disease is influenced by the fats in the habitual diet

### *Serum cholesterol and the incidence of coronary heart disease*

Having disposed of the evidence from wartime experience to his satisfaction Werko states Another corner stone in the lipid-heart theory is the straight line relationship between serum cholesterol and the later incidence of clinical manifestations of CHD Repeatedly it has been stressed that the lower the serum cholesterol the lower the risk and cites 17 references including major publications by the writer on the long term follow up of Minnesota business and professional men and on the Seven Countries Study

*Werko apparently does not understand that the* straight line relationship indicated in most of those studies is a necessary reflection of the mathematical model used in the analyses The simplest relation between two variables is linear of course and in the effort to analyze and present their findings most simply the investigators have generally asked and responded to the questions What is the regression of the incidence of the disease on the concentration of cholesterol in the serum? And when they have moved to multivariate analysis they have examined the multiple regression or have used the multiple logistic equation in which again the question is What is the linear relationship of the incidence to the serum cholesterol level the refinement being in the provision for allowing for interrelations with other variables and emphasis on the statistical significance of the calculated regression coefficient?

### *The Pooling Project*

The results of most of the investigations in this field have been succinctly summarized in terms of a linear regression the numbers of persons and cases are generally too limited to warrant examination of more complex relationships that could equally well be entertained The larger numbers in the Pooling Project combining a number of relatively comparable follow up studies were analyzed both by solutions to the multiple logistic equation and by the distribution of the cases who developed the disease into the quintiles of the distribution of all men for the several variables

Werko states that the final report of the Pooling Project shows that the standard incidence ratio in all studies and in pool 5 was higher in the lowest quintile of serum cholesterol than in the next and goes on to state Even though analyzed in a variety of ways the lack of linearity cannot be obscured Needless to say the authors—all protagonists of the diet–heart disease theory—do not emphasize this remarkable result but say it should lead to further studies Werko makes no mention of the standard errors concerned these show no statistical significance between the rates in the lower two quintiles of the cholesterol distribution Nor does he mention the results of analysis with the logistic equation With the univariate function disregarding age and all other variables the incidence rate in the lowest quintile of cholesterol risk was higher but not significantly so than in the second quintile in three of the four age groups (Tables 22 A–22 D) *However with the bivariate function where serum* cholesterol and age are the two independent variables the rates per 1 000 men per 8 6 years in the successive quintiles are 42 3 58 7 87 4 111 0 and 154 4

Werko states that the Pooling Project did not show any benefit with serum cholesterol below 240 mg/dl In actual fact taking standard incidence for all men as 100 the incidence for men with cholesterol levels under 218 was 66 5 men with entry levels 218–240 had incidence 78 those with cholesterol 240–268 had incidence 129 and the men with entry cholesterol values over 268 had incidence 158 (Table 19) *Werko grudgingly seems to concede that*

very high serum cholesterol levels are undesirable but even if we disregard the men in the top quintile of serum cholesterol we find that the men with cholesterol levels under 240 had only 54% of the incidence of the other men Does that show no benefit ?

*The diet–lipid–coronary heart disease theory*

It is time to say what is meant by the theory of a relationship between the development of coronary heart disease and the lipids especially cholesterol in the blood plasma and in turn to the diet Since it is agreed that clinical coronary heart disease is commonly an end result of many years of atherogenesis it is understood that if the concentration of cholesterol in the plasma is influential it must be considered in terms of many years of action In prospective studies such as those in the Pooling Project the entry concentration is taken to represent the previous life of the person as well as the years of the follow up Obviously any true relationship between the appearance of the clinical disease and the cholesterol level in all previous years must be obscured by the fact that the entry cholesterol level is necessarily subject to much error if it is taken to represent the total exposure The diet might seem simpler because it is possible to make a reasonably good prediction of the effect of serum cholesterol of a given change in the fats and cholesterol in the diet (14) and the average cholesterol concentration in population samples tends to correspond fairly well with the average diets of those populations (3 11 16 23) That relationships are found in spite of these difficulties means that the relationships are basically strong enough to resist complete obliteration by the welter of error attendant on each measurement

The diet–lipid–heart disease theory does not specify a particular mathematical relationship any more than the theory of a relationship between the incidence of cerebrovascular disease and the arterial blood pressure specifies the mathematical form of the relationship As mentioned earlier linear regression analysis has been a popular and useful tool in examining data on the diet serum cholesterol and coronary heart disease but no responsible investigator has suggested that the theory demands a linear relationship As a matter of fact most of us who have long worked in this field have speculated about the possibility that below some critical level the concentration of cholesterol is not a risk factor Werko writes that Stamler maintains that the ideal serum cholesterol should be way below 200 mg/dl In the publication of Stamler that Werko cites I find no such statement about ideal serum cholesterol In my file of reprints from Stamler I also fail to find any statement that corresponds to Werko s charge

Werko s view that the concentration of cholesterol in the serum is a risk factor only at extremely high levels is denied by the findings in every major investigation but it is proper to ask at what level it tends to become important The cholesterol chapter of the report on the 10-year experience of the Seven Countries Study states From the evidence it appears that the serum cholesterol concentration is an important risk factor for the incidence of coronary heart disease at levels of perhaps 220 mg/dl or more (12) Further the summary chapter states on p 325 For coronary deaths no relationship to serum cholesterol is indicated at levels below about 230 or 240 mg/dl These statements refer to over 12 000 men who were aged 40–59 at the start of the 10-year follow up There are no comparable data on younger men but I have a strong impression that a man in his twenties with a cholesterol level over 20[illegible] is at undue risk of coronary disease in the future Long ago we found in Minnesota that among healthy men the mean serum cholesterol for men in the twenties is some 40–50 mg/dl lower than that of men in middle age (13) Later surveys on men of different ages in western populations find similar age trends (2)

*Self selection and bias in prospective studies*

Studies in medical science seldom if ever cover samples proved to be representative of a whole population or even of persons of specified age and sex in the population The same limitation applies to experiments on animals as well as on man to clinical studies to epidemiological surveys and to prospective studies The best we can do is to seek subjects for whom there is no evidence that they are unrepresentative or peculiar in regard to the variables of interest Everyone with any comprehension of the scientific method should be aware of this limitation of the subjects and patients in all medical studies Why then does Werko make such a point about the non responders and drop-outs in the Framingham and similar studies? It is not true as Werko states that the results in the Framingham

study are always said to represent the white US population I have never seen such a statement from Framingham or any of the other major prospective studies But Werko goes on to state categorically that the Framingham results do *not* represent American white people His implication of course is that the relationships found between the incidence of disease at Framingham and the several risk factors may apply to that sample but there is no reason to believe they apply to other people Before making that charge the elementary requirement is that Werko must show that the relationships found at Framingham do not exist in other independent samples

Werko repeatedly talks about this bias in the extensive American population studies What is his evidence of bias in respect to the variables of interest in those studies? Does he take the absence of proof that the samples are truly representative of all mankind to mean that the samples are necessarily biased? In that case practically every medical study including Werko s should be disregarded because there is no proof that the samples were representative Did the animals represent all animals of the species? Did the patients represent all patients in Sweden? Were the controls representative of all healthy Swedes? But Werko goes on to speak of the more carefully conducted European studies He should specify those studies

It is true that only around two thirds of the men invited at Framingham came in for the entry examinations That would invalidate estimation of the prevalence of disease in Framingham at that time But then the prevalence cases were omitted from the further analysis of incidence and the question is whether there is any reason to believe that those clinically healthy persons were different on the average metabolically physiologically anatomically and in personal habits from other persons of the same age sex race and occupation in that part of the world If Werko thinks they are different he should produce some evidence But doubts on this score can hardly persist in the face of the facts that the results in all of these follow up studies in Framingham Minnesota Chicago Albany Los Angeles Tecumseh and the railroad men in the northwestern states are in essential agreement in regard to the big four risk factors—age arterial blood pressure serum cholesterol and cigarette smoking In regard to Werko s view that the serum cholesterol level is unimportant except perhaps at the extreme we note that other major follow up studies are also in general agreement with those mentioned above—the Western Collaborative Group Study (20) the Evans Country Study (26) and the 10-year Oslo study (30) Even the experience of the men of Gothenburg born in 1913 is in general agreement among men in the lower 5 deciles of cholesterol 7 had a myocardial infarction or died from coronary heart disease while 29 men in the upper deciles suffered these fates (25)

Werko states that Stamler turns to using the Pooling Project data in his argument after his own study of the Chicago Gas employees has demonstrated that there is very little difference in CHD incidence in relation to the original serum cholesterol value and Werko cites the final report of the Pooling Project to prove his point So what was the experience of Stamler s Gas Company men as given in the final report of the Pooling Project (18)? With the standardized incidence rate for all Stamler s subjects as 100 the rate for the men with entry cholesterol values under 240 was 83 while that for his men with cholesterol values of 240 or more was 121 for the 5 pool the figures were 81 and 125 5 not significantly different from the Gas Company men But Werko would say that there is very little difference between rates of 83 and 121

### *The clofibrate trial*

Werko seeks to add weight to his attack on the diet–lipids–coronary heart disease hypothesis by dwelling at length on the results of the clofibrate trial The men in the trial who received clofibrate showed an average of a decrease of 9% in serum cholesterol They did not differ from the controls in the incidence of coronary death or angina pectoris but had a significantly lower incidence of non fatal myocardial infarctions Werko rightly concludes that the results are not favorable to the idea of using the drug in the hope of primary prevention especially as overall all-causes mortality of the clofibrate treated men was not reduced But what does this have to do with the hypothesis we are discussing? A fall of 9% in cholesterol is trivial and that was induced by a drug with various metabolic effects not by diet Moreover the clofibrate experiment shares the same serious limitation of all intervention trials to date It began late in the life history of atherogenesis of the subjects and covered only a tenth of their life time to the termination date

teriosclerosis of coronary arteries in sudden unexpected deaths Circulation (Suppl) III 27 1975
18 Pooling Project Research Group Relationship of blood pressure serum cholesterol smoking habit relative weight and ECG abnormalities to incidence of major coronary events final report of the Pooling Project J Chron Dis 31 201 1978
19 Prineas R J & Blackburn H (ed ) Sudden coronary death outside hospital Am Heart Assoc Monograph 47 1975 and Circulation (Suppl) III 1–287 1975
20 Rosenman R H et al Coronary heart disease in western collaborative group study final follow up in 8½ years JAMA 233 872 1975
21 Schettler G Lipoidstoffwechsel und Arteriosklerose Verh Dtsch Ges Inn Med 59 194 1953
22 Schronagel H E The connection between nutrition and mortality from coronary sclerosis during and after World War II Docum Med Geogr Trop (Amst) 5 173 1953
23 Stamler J Population studies In Nutrition lipids and coronary heart disease (ed R I Levy B M Rifkind B H Dennis and N D Ernst) pp 22–28 Raven Press New York 1979
24 Strom A & Jensen R A Mortality from circulatory disorders in Norway Lancet I 126 1951
25 Tibblin G Risk factors for developing myocardial infarction and other diseases The Men born in 1913 study In Preventive cardiology (ed G Tibblin A Keys & L Werko) pp 33–42 Wiley and Sons New York London Sidney and Toronto 1972
26 Tyroler H A Heyden S Bartel A Cassel J Cornoni J C Hames C G & Kleinbaum D Blood pressure and cholesterol as coronary heart disease risk factors Arch Intern Med 128 907 1971
27 Van Unnik J & Straub M Frequency of thrombosis and pulmonary embolism in east and west Docum Med Geogr Trop (Amst) 5 261 1953
28 Vartiainen I Wartime and the mortality of certain diseases in Finland Ann Med Intern Fenn 35 234 1946
29 Vartiainen I & Kanerva K Arteriosclerosis and wartime Ann Med Intern Fenn 36 748 1947
30 Westlund K & Nicolaysen R Ten year mortality related to serum cholesterol Scand J Clin Lab Invest (Suppl) 127 1972
31 Werko L Diet lipids and heart attacks Acta Med Scand 206 435 1979

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# Marked Decrease in Serum HDL Cholesterol Level during Acute Myocardial Infarction

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ABSTRACT The concentrations of serum total and HDL cholesterol and triglycerides were determined in 57 patients during the course of AMI In seven days the concentration of serum cholesterol decreased by 24% and that of HDL cholesterol by 31% The mean HDL/total cholesterol ratio decreased significantly ($p<0.01$) from 0 163 to 0 145 The magnitude of the change in both HDL and total cholesterol showed a positive correlation with infarction size The concentration of triglycerides decreased in seven days on the average by 31% but there was great individual variation, which was not dependent on infarction size Four months after infarction both HDL and total cholesterol as well as triglyceride concentrations had returned to the initial levels There was a significant negative correlation between the concentrations of HDL cholesterol and triglycerides on admission ($r = -0.66$) and after four months ($r = -0.53$) but no correlation after seven days The results indicate that the determination of serum lipids including HDL cholesterol, in patients with AMI can, and should be performed on admission to hospital and not at the time of discharge, in order to get reliable estimates of these cardiovascular risk factors

*Key words* HDL cholesterol lipoproteins myocardial infarction serum cholesterol serum triglycerides

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Acute myocardial infarction (AMI) is associated with several hormonal and metabolic disturbances which are reflected as changes in the concentrations of many serum components (22) For practical purposes it is important to know how these disturbances modify the estimates of various cardiovascular risk factors This applies especially in epidemiological studies and normal clinical routine if dietary advice is given to the patient for possible secondary prevention of AMI

Many authors have described a significant decrease in serum total and low density lipoprotein (LDL) cholesterol levels during the days immediately after infarction followed by a return to the initial level after one or three months (2 4 8 9 15 16 21 24) There is also suggestive evidence of similar behaviour for high density lipoprotein (HDL) cholesterol the rediscovered negative cardiovascular risk factor during AMI (2 8) Data concerning the behaviour of triglycerides conflict (2 10 16 18)

We have examined the behaviour of serum HDL cholesterol concentration during the course of AMI and related it to the changes in serum cholesterol and triglyceride concentrations

## PATIENTS AND METHODS

*Infarction patients*

Fifty seven consecutive unselected patients with AMI 46 men and 11 women admitted to the Coronary Care Unit of our hospital were included in this study Their mean age was 58 years (range 33-77) All patients had typical chest pain unequivocal ECG signs of infarction and elevated serum enzymes The infarction was the first in 40 the second in 16 and the third in one of the patients The localization of infarction was anterior in 37 (65%) and inferior in 20 (35%) of the cases Six patients were diabetics Most patients ($n=32$) had had no medication prior to admission None had hypolipidemic therapy before or after the infarction Eight patients used $\beta$ blocking agents and ten had been digitalized before the infarction

In hospital the patients were kept at bed rest usually for 3-5 days They received the ordinary hospital diet and were not given any special dietary advice during the study Left ventricular failure requiring therapy with diuretics and possibly with digoxin was present in half of the patients ($n=28$) Seven patients died during the first

*Abbreviations* AMI = acute myocardial infarction ASAT aspartate aminotransferase HDL = high density lipoprotein LD = lactate dehydrogenase LDL = low density lipoprotein VLDL = very low density lipoprotein

Table 1 Ser t t tal cl lester l a d HDL cl lester l ( 1 ol/l) d HDL cl lester l/t tal cl lester rat o in AMI pat ents a d c ntr ls ( ean ± S D )

| | Day 1 | Day 7 | 4 months |
|---|---|---|---|
| *Infarct on pa ents* | | | |
| Serum cholesterol | 6 6+1 6 (n=57) | 5 0+1 1 (n=47) | 6 7+1 3 ★ ★ ★ (n=38) |
| HDL cholesterol | 1 01 0 34 (n=53) | 0 69+0 23 * (n=44) | 1 08+0 38 ★ ★ ★ (n=37) |
| HDL cholesterol/total chole terol | 0 163+0 064 (n=53) | 0 145 0 047 * (n=43) | 0 165+0 06? ★ ★ (n=37) |
| Serum trigl cerides | ? 15+1 74 (n 40) | 1 49 0 48* (n=48) | 1 95+0 75 ★ ★ (n=38) |
| *C n rol pa ents* | | | |
| Serum cholesterol | 6 7+1 5 (n=10) | 6 3+1 1 (n=10) | |
| HDL choles erol | 0 98 0 ?4 (n=10) | 0 93+0 18 (n 10) | |
| HDL cholesterol/total cholesterol | 0 154+0 051 (n 10) | 0 15?+0 038 (n=10) | |

*p<0 05 **p<0 01 * *p<0 001 between day 1 and day 7 paired t test (47 pairs for serum total cholesterol 40 pa rs f HDL cholesterol 39 pairs for HDL cholesterol/total cholesterol rat o 33 pa rs for trigl cerides)
★ ★p<0 01 ★ ★ ★p<0 001 between day 7 and 4 mon hs paired t test (35 pa rs for serum total cholesterol 31 pai for HDL cholesterol 31 pairs for HDL cholesterol/total cholesterol ratio 37 pairs for trigl cerides)

week and another se en during the following four months after infarction

*Con r l pa ents*

The control series cons sted of ten pat ents who were hosp tal zed for prolonged angina pectoris but had no in farction They were all at bed rest for 3–5 days after adm ssion

*Cl e cal meth ods*

Blood samples for determ nation of serum l pids were taken on admission (w thin ? hours) and after a 1? hour fast 7 days and 4 months after the onset of nfarction Samples on days 1 and 7 were taken after at least 30 m n recumbency and samples at four months after 30 min n the s tt ng posture Serum cholesterol was determ ned enzymatically (5) Very low dens ty l poprote n (VLDL) and LDL were p ec p tated by a polyethylene glycol solu tion (PEG-6000 final concentration 1? ) (23) and HDL cholesterol was measured from the supernatant Serum trigl cerides were determined acco d ng to Royer and Ko (17) w th a Technicon Autoanalyzer* The s ze of the infarction was estimated from the maximum serum aspar tate aminotransferase (ASAT) and lacta e dehydrogenase (LD) values Samples for ASAT measurement were drawn 0 6 1? ?4 ?? and 120 hou s and for LD measure ment 0 ?4 7? and 120 hours after adm s ion

*Stat st cal methods*

Paired t test was used to compare value from one and the same pat ent at various points of t me Student s t test was used to compare the mean values of different group Correlat on coeff c ents were calculated us ng l near corr lation (comparison between values from day 1 and fro the 4-month sample) or exponential regress on (compa son between HDL cholesterol and triglycerides)

## RESULTS

*Infarct on pat ents*

The mean serum cholesterol concentration on a mission was with n normal limits 6 6 mmol/l (6 for men and 7 8 for women) and decreased b ?4 4% during the following week (Tables I and II The concentration of HDL cholesterol was alread low 1 02 mmol/l (0 98 for men and 1 16 for wome our mean level for healthy men is about 1 4 mmol/l on adm ssion and decreased by a h gher percenta (31 0%) than serum total cholesterol The HDL/t tal cholesterol ratio was therefore sign ficantly lo er (p<0 01) after one week than on admission

The concentration of triglycerides decrease during the first week of infarct on by an average 31% but the ind vidual variation was great som pat ents even showing an increase The behav ou of all serum l p ds measured was sim lar in diabet c and other patients during infarction (data not

Table II *Decre se i ser i l p ds d r ng tl e f rst week of AMI ( rean + S D )*

| | Serum cholesterol | | HDL cholesterol | | LDL | STG |
|---|---|---|---|---|---|---|
| | Decrease (%) | n | Decrease (%) | n | | |
| Whole series | 4 4+15 7 | 47 | 31 0+16 7 | 40 | | |
| Sex | | | | | | |
| Men | 23 8+16 0 | 40 | 9 4+16 4 | 35 | | |
| | NS | | NS | | | |
| Women | 6 9±1 8 | 7 | 41 8+16 4 | 5 | | |
| Age (y ) | | | | | | |
| ≤50 | 0 3+12 3 | 12 | 8 5+11 1 | 10 | | |
| | NS | | NS | | | |
| 51–60 | NS 23 0+17 7 | 19 | 33 5±19 6 | 16 | NS | |
| | NS | | NS | | | |
| >60 | 8 8+14 5 | 16 | 9 8 17 2 | 14 | | |
| Heart failure[a] | | | | | | |
| Yes | 9 5+14 9 | 0 | 37 8+18 4 | 18 | | |
| | $p<0\ 05$ | | $p<0\ 01$ | | | |
| No | 0 4+15 0 | 27 | 24 7+11 7 | 22 | | |
| Initial serum cholesterol (mmol/l)[b] | | | | | | |
| >7 5 | 34 2+8 4 | 13 | 37 4 17 0 | 10 | | |
| | $p<0\ 01$ | | NS | | | |
| <7 5 | 0 5 16 0 | 34 | 28 8+16 3 | 30 | | |

[a] Max mum ASAT in pat ents w th heart failure 5 43 2 97 and in pat ents w thout heart failure 3 35+1 87 μkat/l ($p<0\ 01$)
[b] The s ze of nfarct on d d not d ffer between the two groups as estimated by highest serum ASAT values

shown) The concentrat ons of total and HDL cholesterol the HDL/total cholesterol ratio and the concentrat on of triglycerides had returned to their n tial values n four months and the values from four months correlated well w th those from adm s s on (respective correlat on coeffic ents r 0 58 $p<0\ 001$ r 0 71 $p<0\ 001$ r 0 81 $p<0\ 001$ r 0.50 $p<0\ 01$)

*Control pat ents*

In control patients the concentrat ons of total and HDL cholesterol on adm ss on d d not d ffer from those of nfarction patients However in control pat ents no s gn ficant decrease had occurred in e ther total or HDL cholesterol at seven days after admission

*Stat st cal correlat s*

The magn tude of the decreases in serum total and HDL cholesterol was not dependent on the sex or age of the pat ents nor on the local zation of nfarc tion but the decrease in total cholesterol was s g n ficantly steeper in pat ents who were init ally hypercholesterolem c The r HDL cholesterol also decreased more than that of others but th s d ffer ence was not stat stically s gn f cant. The decrease in both total and HDL cholesterol correlated posi tively (r 0 31–0 34 $p<0\ 05$ and r 0 33–0 34 $p<0\ 05$ respectively) with the s ze of infarct on (Fig 1) The decrease in both parameters was more marked in pat ents who developed heart failure but the r infarct on s ze was sign ficantly larger than that of those w thout heart fa lure

The decrease in serum triglycerides in the acute phase was not dependent on the sex or age of the pat ents or on the local zation and size of infarct on or on the in tial cholesterol level On day one and after four months there was a sign f cant negative correlation between the concentrat ons of serum triglycerides and HDL cholesterol but no corre lat on was present seven days after adm ss on (F g 2)

## DISCUSSION

Several stud es have shown a sharp decrease in serum total and LDL cholesterol during the first days of AMI followed by a return to the in t al level

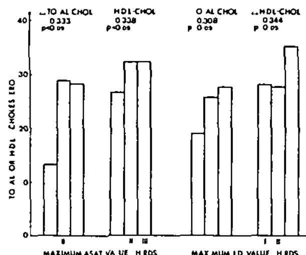



*Fig 1* Relation between infarction size and change in serum total and HDL cholesterol concentrations during the first week of AMI The size of infarction was estimated from maximum serum ASAT (normal value ≤0 58 μkat/l) and LD (normal value ≤7 3 μkat/l) values The patient series (*n* 57) was divided into thirds for ASAT I<2 67 μkat/l (mean 1 60) II 2 67–4 83 (mean 3 70) III>4 83 (mean 6 77) for LD I<19 2 μkat/l (mean 13 7) II–19 2 30 8 (mean 25 3) III>30 8 (mean 48 2)

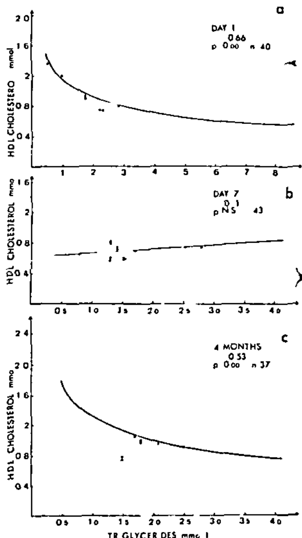



*Fig 2* Relation between serum triglycerides and HDL cholesterol during myocardial infarction The equations for the correlations are (*a*) y 0 0473×10 ... (*b*) y 0 0527*x* + 0 672 (*c*) y 0 1702×10 ...

in 1–3 months (2 4 8 9 13 15 16 21 24) Thereafter no further changes have been observed (11 21) Determinations made within 24 hours or after three months from the onset of infarction are therefore reliable for screening of dyslipoproteinemias (15) The decrease in serum cholesterol in the early phase is a sequence of infarction and not of bed rest and its magnitude is positively correlated with infarction size (24) The decrease is not dependent on sex age arrhythmias medication or development of heart failure (15) A similar decrease in serum β lipoproteins occurs after major surgery suggesting that the phenomenon is unspecifically associated with tissue injury (25)

Less attention has been paid to the behaviour of HDL during myocardial infarction Miettinen (14) found a statistically significant decrease during the first week of infarction in serum α lipoprotein cholesterol using paper electrophoresis Johansson et al (12) and Ritland et al (16) using an immunodiffusion method showed a clearcut decrease in serum apolipoprotein A 5–8 days after infarction followed by a return to the initial level in 3–7 weeks Avogaro et al (2) found a sharp decrease during AMI in apoprotein A I the main protein component of HDL the lowest value occurring 7 days after infarction They also measured the concentration of serum HDL cholesterol by the heparin manganese precipitation method but were unable to show a statistically significant decrease

We found that the HDL cholesterol concentration which is easier to measure than HDL apoproteins behaved almost similarly to serum total cholesterol concentration during the course of infarction but the decrease in HDL cholesterol after one week was even sharper than that of total cholesterol The decrease in both total and HDL cholesterol was positively correlated with infarct on size as reported earlier for serum cholesterol (24) In keeping with the previous finding for serum

cholesterol (15) we found no correlation between sex or age of patients and the decrease in HDL and total cholesterol levels We did not study the mechanism of the decrease in HDL cholesterol but it is probable that the decrease is unspecifically related to various tissue injuries since a decrease in $\alpha$ lipoproteins similar to that for HDL cholesterol in the present study occurs in inflammatory diseases and after major surgery (25)

Earlier studies have suggested either an increase (2 18 19) decrease (3 10) or no clearcut change (7 13 16) in serum triglyceride concentrations in the acute phase of an AMI Our results show a trend towards a decrease but there was great individual variation without any evident explanation Normally a negative correlation exists between the concentrations of serum triglycerides and HDL cholesterol (1 6) This was also the case in our study on day 1 and after four months The absence of such a negative correlation after seven days suggests that not only the absolute values of serum lipids but also their interrelationships are thoroughly disturbed in the early phase of an AMI

Because the values for all lipids studied—HDL and total cholesterol and triglycerides—returned to their initial level after four months values from the first two hours can be regarded as reliable Since these samples were taken without systematic previous fasting the values for triglycerides are probably in some cases too high However taking the first samples during the first 36 hours of an AMI after a 12 hour fast as suggested by Fyfe et al (10) may give too low HDL and total cholesterol values Theoretically because the samples on days 1 and 7 in our study were taken in recumbent position and the samples after four months in a sitting posture there should be an increase of about 5% in the last values due to hemoconcentration (20) Actually this was the case for HDL cholesterol Determinations at the time of discharge nowadays usually 10–12 days after infarction or even earlier give an erroneous idea of the concentrations of these lipids and also of their relationships This is especially important in the case of hypercholesterolemic patients and patients with large infarctions Therefore we feel that determinations of serum total and HDL cholesterol as well as triglycerides should be made immediately after admission to hospital This yields reliable values and any secondary prevention can be started while the patient is in hospital at a time when he is most impressionable

## REFERENCES


1 Albers J J Cheung M C & Hazzard W R High-density lipoproteins in myocardial infarction survivors Metabolism 27 479 1978

2 Avogaro P Bittolo Bon G Cazzolato G Quinci G B Sanson A Sparla M Zagatti G C & Caturelli G Variations in apolipoproteins B and $A_1$ during the course of myocardial infarction Eur J Clin Invest 8 121 1978

3 Besterman E M M The effects of acute cardiac infarction and of heparin therapy on the lipoproteins Br Heart J 20 21 1958

4 Biorck G Blomqvist G & Sievers J Cholesterol values in patients with myocardial infarction and in a normal control group Acta Med Scand 156 493 1957

5 Boehringer Mannheim GmbH CHOD-PAP method for Technicon Autoanalyzer®

6 Carlson L A & Ericsson M Quantitative and qualitative serum lipoprotein analysis Part 2 Studies in male survivors of myocardial infarction Atherosclerosis 21 435 1975

7 Deegan T & Hayward P J Serum lipid changes following myocardial infarction J Atheroscler Res 5 267 1965

8 Dodds C & Mills G L Influence of myocardial infarction on plasma lipoprotein concentration Lancet I 1160 1959

9 Enger S C & Ritland S Serum lipoprotein pattern in myocardial infarction Acta Med Scand 187 365 1970

10 Fyfe T Baxter R H Cochran K M & Booth E M Plasma lipid changes after myocardial infarction Lancet 2 997 1971

11 Gustafson A Elmfeldt D Wilhelmsen L & Tibblin G Serum lipids and lipoproteins in men after myocardial infarction compared with representative population sample Circulation 46 709 1972

12 Johansson B G Kindmark C O Trell E Y & Wollheim F A Sequential changes of plasma proteins after myocardial infarction Scand J Clin Lab Invest (Suppl) 124 117 1972

13 Kirkeby K Disturbances in serum lipids and in their fatty acid composition following acute myocardial infarction Acta Med Scand 192 523 1972

14 Miettinen M Serial studies of serum lipids and lipoproteins in acute myocardial infarction Ann Med Int Fenn 46 69 1957

15 Mundy G R & McPherson D G Variations in serum cholesterol levels after myocardial infarction Med J Aust 1 278 1973

16 Ritland S Blomhoff J P Enger S C Skrede S & Gjone E The esterification of cholesterol in plasma after acute myocardial infarction Scand J Clin Lab Invest 35 181 1975

17 Royer M E & Ko H A simplified semiautomated assay for plasma triglycerides Anal Biochem 29 405 1969

18 Smith E B Lipoprotein patterns in myocardial infarction Relationship between the components identified by paper electrophoresis and in the ultracentrifuge Lancet 2 910 1957

19 Snyder S Durham B C Iskandrian A S Coodley E L & Linhart J W Serum l p ds and glycoproteins in acute myocardial infarct on Am Heart J 90 582 1975
20 Tan M H Wilmshurst E G Gleason R E & Soeldner J S Effect of posture on serum l p ds N Engl J Med 289 416 1973
21 Tibbl n G & Cramér K Serum l p ds during the course of an acute myocardial infarction and one year afterwards Acta Med Scand 174 451 1963
22 Vetter N J Strange R C Adams W & Oliver M F Initial metabol c and hormonal response to acute myocardial infarction Lancet 1 284 1974
23 Vikari J Precipitation of plasma l poprote ns by PEG 6000 and its evaluat on with electrophores s and ultracentrifugat on Scand J Cl n Lab Invest 36 265 1976
24 Watson W C Buchanan K D & Dickson C Serum cholesterol levels after myocard al infarct on Br Med J 2 709 1963
25 Werner M Serum prote n changes during the acute phase reaction Clin Chim Acta 25 299 1969

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# Established Beta-Adrenergic Receptor Blocking Therapy and Acute Myocardial Infarction

## *A Clinical Study of Risks and Benefits*

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**ABSTRACT In order to evaluate the risks and benefits of continuing established therapy with β adrenergic receptor blocking drugs during acute myocardial infarction (AMI) 183 consecutive patients, 63 with (β blocker group) and 120 without (control group) this therapy, were studied Detailed information on previous diseases present symptoms, established medication, clinical and laboratory findings on admission and during the first 12 hours in the CCU was collected The incidences of congestive heart failure, hypotension, AV blocks and ventricular arrhythmias were not significantly different in the two groups The incidence of anterior wall infarction was the same in both groups (38%) but inferior wall infarction was significantly more common in the control group (8 vs 28%, $p<0.01$) Thus, continuation of established therapy with β adrenergic receptor blocking drugs does not seem to increase the risk of complications after hospital admission for AMI The reason for the low incidence of inferior wall infarction in the β-blocker group is not clear but it cannot be excluded that when patients on β adrenergic receptor blocking therapy develop an inferior AMI, they may run a greater risk of sudden death**

*Key words* β receptor blockade acute myocardial infarction side effects

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Beta adrenergic receptor blocking drugs are now used extensively for treatment of a wide variety of diseases e g angina pectoris cardiac arrhythmias and hypertension It has also been suggested that these agents should be used routinely after myocardial infarction in order to reduce the high mortality in this patient group (1 6 10) However treatment with β adrenergic receptor blocking agents might involve a risk of adverse reactions e g bradyarrhythmias heart block congestive heart failure and hypotension This risk would be especially high in patients developing acute myocardial infarction (AMI) during long term therapy with these drugs as the infarction per se frequently causes the same complications Also it has been questioned if β adrenergic receptor blocking therapy could be potentially hazardous during the first month after the acute event in patients with inferior wall infarctions (6 7) Therefore we found it of interest to study the risks and benefits of continuing established β adrenergic receptor blocking therapy in patients with verified AMI

## PATIENTS AND METHODS

All patients admitted to the coronary care unit (CCU) Regional Hospital Linkoping because of suspected or verified AMI were studied during the first 12 hours in the CCU A special data form was filled in for each patient with basic information (age sex previous diseases medication before admission with special reference to type and dose of β adrenergic receptor blocking drug symptoms and onset clinical findings on admission etc ) Clinical course laboratory data ECG findings and the final diagnosis were also registered on the same data sheet

All patients were under standard CCU surveillance by the ordinary medical and nursing staff and received the same basic therapy Blood pressure respiratory rate and other signs of heart failure (pulmonary oedema basal crepitations supraventricular tachycardia etc ) were registered at least every 3 hours On admission and on each of the two following days blood samples were drawn for determination of serum levels of aspartate amino transferase alanine aminotransferase and lactate dehydrogenase Blood samples for determination of creatine phosphokinase (CK) were drawn on admission and thereafter every 6 hours for at least 24 hours The maximum value of CK was used for the estimation of infarction size

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*Abbreviations* AMI = acute myocardial infarction CCU = coronary care unit CK = creatine phosphokinase

Table I *Patient characteristics on admission*

| | Beta blocker group | | Control group | | |
|---|---|---|---|---|---|
| | N | % | N | % | p |
| Age (y) | | | | | |
| <50 | 4 | 6 | 11 | 9 | N S |
| 50–59 | 16 | 25 | 28 | 23 | N S |
| 60–69 | 27 | 43 | 52 | 43 | N S |
| 70–79 | 15 | 24 | 27 | 23 | N S |
| ≥80 | 1 | 2 | 2 | 2 | N S |
| Sex | | | | | |
| Male | 45 | 71 | 87 | 73 | N S |
| Female | 18 | 29 | 33 | 27 | N S |
| Previous myocardial infarction | | | | | |
| None | 23 | 37 | 84 | 70 | <0 001 |
| 1 | 19 | 30 | 28 | 23 | N S |
| 2 and more | 21 | 33 | 8 | 7 | <0 001 |
| History of angina pectoris | 52 | 83 | 48 | 40 | <0 001 |
| History of hypertension | 36 | 57 | 31 | 26 | <0 001 |
| Maintenance therapy with | | | | | |
| Digitalis | 33 | 52 | 36 | 30 | <0 01 |
| Diuretics | 25 | 40 | 32 | 27 | N S |
| Antihypertensive drugs[b] | 21 | 33 | 14 | 12 | N S |
| Type of $\beta$-blocker | | | | | |
| Propranolol | 18 | 29 | – | – | |
| Alprenolol | 19 | 30 | – | – | |
| Metoprolol | 22 | 35 | – | – | |
| Other | 4 | 6 | – | – | |
| Admission delay (h) | | | | | |
| <2 0 | 14 | 22 | 24 | 20 | N S |
| 2 0– 5 9 | 31 | 49 | 43 | 36 | N S |
| 6 0–11 9 | 9 | 14 | 25 | 21 | N S |
| 12 0–23 9 | 6 | 10 | 19 | 16 | N S |
| ≥24 | 3 | 5 | 9 | 8 | N S |

[a] During 1 month before admission
[b] Other than diuretics and $\beta$-adrenergic receptor blocking agents

The diagnosis of AMI was based on at least two of the following conventional criteria 1) History of central chest pain of at least 15 min duration 2) Sequential and localized ECG changes in ST segments and T waves and/or development of Q waves 3) Appropriate enzyme elevations 4) Autopsy findings of myocardial necrosis of an age corresponding to the onset of symptoms

*Electrocardiographic recordings and analysis*

Routine 12 lead ECGs were registered upon admission and then daily for at least the next three days The site of infarction was judged by the ordinary CCU medical staff from the changes observed in these daily ECGs During the 12 hours of the study the monitored ECG was recorded continuously on an 8-channel ink jet ECG recorder at a paper speed of 10 mm/sec All ECG recordings were analysed by one of the authors The various types of arrhythmias were classified minute by minute according to conventional criteria. Ventricular tachycardia is defined as 3 or more consecutive ventricular ectopic beats at a ra[te] of 120 beats/min or more

*Statistical methods*

The differences between patient groups were analys[ed] with respect to relative numbers by the $\chi^2$ test Wh[en] there was only a small number of observations Fishe[r's] exact test was used

## RESULTS

*Patient characteristics*

During a 6-month period Jan –July 1978 a total [of] 405 patients were admitted to the CCU because [of] suspected AMI In 183 patients (45%) the su[s]pected diagnosis of AMI was confirmed Only th[e] results from these patients will be reported

Table II *Clinical and laboratory findings during study period and during stay in hospital $CK_{max}$ and infarction site*

| | β blocker group | | Control group | | |
|---|---|---|---|---|---|
| | N | % | N | % | p |
| Signs of congestive heart failure | 26 | 41 | 49 | 41 | N S |
| Hypotension | 5 | 8 | 10 | 8 | N S |
| Bradyarrhythmia[b] | 8 | 13 | 6 | 5 | N S |
| AV block II + III | 3 | 5 | 10 | 8 | N S |
| Ventricular arrhythmia[c] | 12 | 19 | 32 | 27 | N S |
| $CK_{max}$ (μkat/l) | | | | | |
| <0 8 | 7 | 11 | 16 | 13 | N S |
| 0 8–5 0 | 36 | 57 | 51 | 43 | N S |
| 5 1–10 0 | 10 | 16 | 29 | 24 | N S |
| >10 0 | 10 | 16 | 24 | 20 | N S |
| Site of infarction | | | | | |
| Anterior wall | 24 | 38 | 45 | 38 | N S |
| Lateral wall | 5 | 8 | 2 | 2 | N S |
| Inferior wall | 5 | 8 | 34 | 28 | <0 01 |
| Anterior wall + lateral wall | 2 | 3 | 4 | 3 | N S |
| Inferior wall + lateral wall | 6 | 10 | 14 | 12 | N S |
| Combined[d] | 5 | 8 | 1 | 1 | N S |
| Uncertain | 16 | 25 | 20 | 17 | N S |

Systolic pressure <90 mmHg
[b] <40 impulses/min without AV block
Ventricular fibrillation ventricular tachycardia (3 or more ventricular ectopic beats (VEB) in sequence and with a rate of 120 beats/min or more) R-on T type VEB
[d] ECG changes over anterior–inferior as well as over anterior–inferior and lateral walls

Of the 183 patients with AMI 63 (34%) were on long term β adrenergic receptor blocking therapy (β blocker group) whilst 120 patients (66%) had no such therapy (control group) Some characteristics of the patients on admission are given in Table I The age and sex distributions were similar in the two groups Also the time between onset of pain and admission to CCU was similar On the other hand a history of two or more previous myocardial infarctions angina pectoris and hypertension was more common in the β blocker group ($p<0\ 001$)

*Complications during study period*

Table II gives the frequency of some complications of AMI and/or side effects to treatment with β-adrenergic receptor blocking drugs No significant differences were found between the two groups as regards any of the registered variables

In 12 (19%) of the 63 patients in the β blocker group this treatment was discontinued because of signs of congestive heart failure hypotension or bradyarrhythmias There was no difference in frequency of complications/side effects between the three most common types of β adrenergic receptor blocking agents in this study (Table III) Of the 183 patients 15 (8%) died during the study period i e during the first 12 hours in the CCU Six of the 8 deaths in the β blocker group and 4 of the 7 deaths in the control group were due to progressive left heart failure

*Infarction size*

The infarction size estimated from the maximum CK value is given in Table II There was no significant difference between the two groups of patients

Table III *Comparison between number of patients treated with the three most common β-adrenergic receptor blocking drugs and complications/side effects*

| | Propranolol (n=19) | Alprenolol (n=19) | Metoprolol (n 21) |
|---|---|---|---|
| Left heart failure | 8 | 9 | 10 |
| Hypotension | 3 | 0 | 2 |
| Bradyarrhythmia | 5 | 1 | 2 |
| AV block II + III | 1 | 0 | 1 |

Table IV *Previous cardiovascular diseases in 108 patients sustaining anterior or inferior AMI*

| | Anterior wall infarction ($n$=69) | | | Inferior wall infarction ($n$=39) | | |
|---|---|---|---|---|---|---|
| | $\beta$ blocker group ($n$=24) | Control group ($n$=45) | $p$ | $\beta$ blocker group ($n$=5) | Control group ($n$=34) | $p$ |
| History of angina pectoris | 21 | 21 | <0 01 | 5 | 11 | <0 05 |
| Previous myocardial infarction | | | | | | |
| 0 | 8 | 32 | <0 01 | 3 | 27 | N S |
| 1 | 8 | 11 | N S | 1 | 5 | N S |
| 2 or more | 8 | 2 | <0 01 | 1 | 2 | N S |
| History of hypertension | 11 | 11 | N S | 3 | 7 | N S |

*Site of infarction*

Table II shows the distribution of infarction sites in the two groups An anterior wall location is exactly as common in the $\beta$ blocker as in the control group On the other hand an inferior wall location is significantly more frequent in the control group (8 vs 28% $p<0 01$) This discrepancy between the two groups is still valid if patients with infarction of the inferior lateral wall are added to those with inferior infarction alone (18 vs 40% $p<0 01$) Adding patients with combined anterior lateral infarction to those with anterior infarction does not however change the equal distribution between the $\beta$ blocker and the control group A comparison between patients on long term therapy with $\beta$ adrenergic receptor blocking drugs who develop anterior and inferior infarction respectively does not indicate any positive differences with respect to the frequency of angina pectoris and hypertension or the number of previous myocardial infarctions (Table IV)

## DISCUSSION

Despite the widespread use of $\beta$ adrenergic receptor blocking agents in the therapy of cardiovascular diseases only one systematic survey of the adverse effects of these drugs has been found in the literature (4) All the patients in that study received propranolol for angina arrhythmias hypertension or thyrotoxicosis Adverse reactions attributed to propranolol were found in 9 4% The results from that survey can hardly be transferred to patients with AMI or treatment with other $\beta$ adrenergic receptor blocking drugs

In the present study the frequency of reactions that possibly could be attributed to the therapy with $\beta$ adrenergic receptor blocking drugs was similar in both groups However the results must be interpreted with great caution because of the study s design It could be that some patients in the control group were not on $\beta$ adrenergic receptor blocking therapy because of a latent congestive heart failure or hypotension On the other hand there were more patients in the $\beta$ blocker group with a history of one or more myocardial infarctions angina pectoris and hypertension Also in this group the frequency of complications attributable to the treatment with $\beta$ adrenergic receptor blocking drugs and/or the AMI itself was not higher than that reported by Henning and Lundman (5) in a similar series of patients not on $\beta$ adrenergic receptor blocking therapy

Fox et al (3) found in a prospective study similar to the present that myocardial infarction occurred significantly more often in a control group than in a $\beta$ blocker group and suggested that established beta blockade may protect some patients from the development of myocardial infarction These results were not confirmed by our study as development of AMI was equally common in both groups Thus 63 (46%) of 136 patients with and 120 (45%) of 269 patients without long term $\beta$ adrenergic blocking therapy developed an AMI after admission to CCU On the other hand the myocardial necrosis as judged from the maximum CK value seems to be somewhat less extensive in the $\beta$ blocker group This finding could support the results from experimental as well as human studies that $\beta$ receptor blocking agents might save jeopardized myocardium after coronary occlusion (2 8 9) However like Fox et al (3) we found that the

mortality rate seemed to be as high in the β blocker as in the control group

The most notable finding in the present study is the small number of patients developing inferior wall infarction in the β blocker group We have not found any obvious explanation for this significant difference between the two groups Thus in the β blocker group a combined history of angina pectoris hypertension and previous myocardial infarction seems to be as frequent among patients developing inferior as anterior myocardial infarction Also there was no difference in age sex or delay between onset of symptoms and admission to hospital

One hypothesis that could explain the small number of patients in the β blocker group who developed inferior wall infarction is that these patients died suddenly and were never admitted to hospital This would support results from the Multicentre International Study (6 7) indicating that β adrenergic receptor blocking therapy could be hazardous in the immediate postinfarction period if the infarct is localized to the inferior wall Further prospective studies are needed to answer this important question

## REFERENCES


1 Ahlmark G Saetre H & Korsgren M Letter Reduction of sudden deaths after myocardial infarction Lancet 2 1563 1974

2 Barber J M Boyle D McC Chaturvedi N C Singh N & Walsh M J Practolol in acute myocardial infarction Acta Med Scand (Suppl) 587 213 1975

3 Fox K M Chopra M P Portal R W & Aber C P Long term beta blockade Possible protection from myocardial infarction Br Med J 1 117 1975

4 Greenblatt D J & Koch Weser J Adverse reactions to propranolol in hospitalized medical patients a report from the Boston Collaborative Drug Surveillance Program Am Heart J 86 478 1973

5 Henning R & Lundman T Swedish co-operative CCU study A study of 2008 patients with acute myocardial infarction from twelve Swedish hospitals with coronary care unit Acta Med Scand (Suppl) 586 1975

6 A Multicentre International Study Improvement in prognosis of myocardial infarction by long term beta adrenoreceptor blockade using practolol Br Med J 3 735 1975

7 — Reduction in mortality after myocardial infarction with long term beta adrenoreceptor blockade Supplementary report Br Med J 2 419 1977

8 Reimer K A Rasmussen M M & Jennings R B On the nature of protection by propranolol against myocardial necrosis after temporary coronary occlusion in dogs Am J Cardiol 37 520 1976

9 Shatney C H MacCarter D J & Lillehei R C Effects of allopurinol propranolol and methylprednisolone on infarct size in experimental myocardial infarction Am J Cardiol 37 572 1976

10 Wilhelmsson C Vedin J A Wilhelmsen L Tibblin G & Werko L Reduction of sudden deaths after myocardial infarction by treatment with alprenolol Lancet 2 1157 1974

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# Interaction of Clonidine and β-Blockers

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**ABSTRACT On the hypothesis that non selective β-blockers can antagonize or reverse the antihypertensive effect of clonidine (C), 12 hypertensive outpatients were treated with C alone and in combination with propranolol (P), atenolol (A) and prazosin (Pz) C alone (0 11 or 0 22 mg b i d ) or in combination with P (80 mg b i d ) did not provide normotension Changing P to A (50 mg b i d ) reduced supine systolic and diastolic pressures which now were significantly lower ($p<0$ 01) than during C alone Changing A to P again resulted in elevated pressures Pz (1 mg t i d ) added to the C+P regimen lowered supine blood pressures to the levels otherwise recorded during C+A C dose-dependently contracted rabbit aortic spiral in vitro reaching about 50% of maximum responses to noradrenaline Pz abolished this response P (0 1–10 μg/ml) but not A somewhat enhanced responses to high doses of C Sotalol rather antagonized C contractions We conclude that A but not P enhances the antihypertensive action of C No hypertensive interaction was observed**

*Key words* interaction clonidine β blockers propranolol atenolol prazosin

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Combined administration of clonidine and β adrenoceptor blocking agents may be deleterious in patients with renal failure (7) and a combination of clonidine and sotalol has been reported to elevate blood pressure (BP) from the pretreatment levels (9) In this study 6 out of 10 hypertensive patients treated with clonidine and sotalol showed higher BP values than those measured after either drug alone Such an unexpected effect can be explained by assuming that clonidine as an α adrenoceptor agonist lowers BP via central action but in periphery it may rather increase vasoconstriction Non selective β blockers when opposing the $\beta_2$ receptor mediated vasodilation also constrict blood vessels (1) and the two drugs together might increase the peripheral resistance

To elucidate this drug interaction we have treated hypertensive outpatients to get answers to the following questions Do β blockers indeed reverse the antihypertensive effect of clonidine to hypertension? Is there a difference between cardioselective atenolol and non selective propranolol in this respect? Do low doses of a vasodilator drug prazosin modify these responses?

In vitro experiments on isolated aortic strips were carried out to elucidate further this drug interaction

## MATERIAL AND METHODS

### *Clinical study*

*Patient selection* The clinical study was carried out in 12 outpatients 8 males and 4 females with established essential hypertension Their ages ranged from 29 to 56 years (mean 44) and their weights from 49 to 100 kg (mean 76) Based on the WHO classification 8 patients had grade I and 4 grade II hypertension Informed written consent was obtained from the patients before entering the study

*Trial design* The design of the study is shown in Fig 1 As part of an earlier trial the patients were treated with clonidine (0 11 or 0 22 mg twice daily) and a diuretic (cyclothiazide 2 5 mg daily) for the last 6 weeks of that trial The diuretic was then withdrawn and the treatment continued with clonidine in the same dose for 3 weeks

The dose of clonidine in the former trial had proved to be 0 11 mg b i d in 4 patients and 0 22 mg b i d in 8 Propranolol 80 mg b i d was then added to the regimen for 3 weeks whereafter it was changed to atenolol 50 mg b i d for another 3 weeks Thereafter atenolol was changed to propranolol 80 mg b i d again Prazosin 1 mg t i d was added to this combination for 3 weeks For the next 3 week period propranolol was withdrawn and the study was finished by treating the patients with clonidine alone The clonidine dose remained unchanged throughout the study The patients received clonidine propranolol and atenolol at 8 a m and 8 p m Prazosin was given at 8 a m 2 p m and 8 p m

Three patients were excluded from the clonidine + propranolol + prazosin phase because of side-effects such as postural hypotension and vertigo When comparing results after prazosin with those after other treatments the data for the 9 patients who completed the trial were used One patient dropped out during the late prazosin + clonidine phase because of poor co-operation

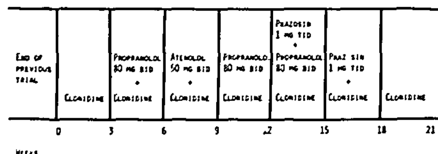



*Fig 1* Design of the trial

*Measurements* The same trained nurse measured BP throughout the study in the same room of the Outpatient Clinic using the same standard mercury sphygmomanometer She was unaware of the treatment of the patients The visits were arranged at the end of each 3-week treatment period at 4–5 p m The BP was measured on the right arm in the supine and standing positions The supine BP was measured after 10 min rest in recumbency and standing BP 2 min after assumption of the erect position Three readings were made in supine position 1 min apart and the corresponding averages were used for the calculation of results The first and fifth phases of Korotkoff sounds were used as criteria for systolic and diastolic pressures respectively Pulse rate was recorded after the BP measurements in supine and standing position for 60 sec by palpation at the wrist

To eliminate non significant side-effects the patients were asked after the BP measurements by the same investigator in an uninterested voice if they had noticed any side effects Patient compliance was estimated by counting the tablets

*Statistical analysis* The paired *t* test was used for comparing the mean values of BP and heart rate (HR)

*In vitro experiments*

Isolated aortic strips of the rabbit were suspended in Krebs bicarbonate solution aerated with 5% $CO_2$ in oxygen Their cumulative responses (5) to noradrenaline and clonidine were recorded in the absence and presence of propranolol atenolol or sotalol concentrations in bath fluid When producing the control dose–response graph the agonist dose was increased 3-fold every 5 min and this was continued up to a maximum response With each preparation four consecutive dose–response graphs were produced starting from control responses and raising the concentration of the β blocker concerned The maximum responses were produced with noradrenaline at the end of each dose–response graph The increase in the maximum response of the tissue during repetitive challenges (5) was taken into account when calculating the responses in per cent of the maximum This was done by producing a series of control dose–response graphs four consecutive times with the same tissue

## RESULTS

*Blood pressure and heart rate* Twelve patients completed the study The changes in mean values for the supine and standing systolic and diastolic BP during the different phases of the study are shown in Table I It appears that combining propranolol with clonidine lowered supine systolic BP ($p<0.05$)

Table I *Systolic (SBP) and diastolic blood pressure (DPB) (mmHg) and heart rate (beats/min) in supine and standing positions during different treatment periods in 12 patients (mean ± S D )*

| Treatment | Supine SBP | Supine DBP | Supine HR | Standing SBP | Standing DBP | Standing HR |
|---|---|---|---|---|---|---|
| Initial | 188±23 | 121±14 | 74± 8 | | | |
| End of previous trial (cyclothiazide + clonidine) | 147±18 | 100± 9 | 69±12 | 145±19 | 105±12 | 79±11 |
| Clonidine | 156±18 | 102±10 | 66± 7 | 150±21 | 108±11 | 77±10 |
| Clonidine + propranolol 80 mg b i d | 148±20 | 99±12ns | 59± 8** | 144±26ns | 105±13ns | 66± 7 |
| Clonidine + atenolol 50 mg b i d | 145±20 * | 94±13 * | 59± 8* | 143±26ns | 102±17ns | 63± 8 * |
| Clonidine + propranolol 80 mg b i d | 151±23ns | 100±12ns | 57± 8 * | 144±23ns | 103±14ns | 62± 9** |
| Clonidine + propranolol 80 mg b i d + prazosin 1 mg t i d * | 147±18* | 96±14* | 54± 8* | 147±16ns | 105±16ns | 63±10 |
| Clonidine + prazosin 1 mg t i d * | 155±16 | 102± 9 | 68±15 | 151±30 | 105±18 | 81±18 |
| Clonidine | 158±19 | 104±11 | 68± 9 | 151±21 | 110±11 | 78±11 |

9 Patients * 11 patients

ns = non-significant *$p<0.05$ * $p<0.01$ compared with values from the first treatment period with clonidine alone

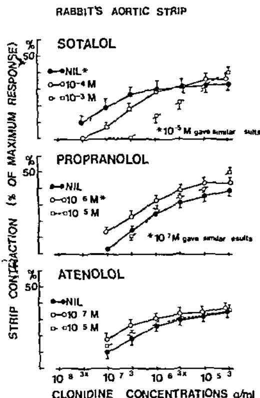



*Fig 2* Cumulative responses of the isolated rabbit's aortic strip to clonidine (mean ± S E of 5–7 strips) The β-blockers were added to the bath fluid 5 min before the first dose of clonidine the other doses of clonidine being added at 5 minute intervals

By contrast the supine diastolic as well as the standing systolic and diastolic BP remained largely unchanged Slowing of the HR was statistically significant ($p<0.01$)

The change of propranolol to atenolol reduced supine systolic and diastolic BP as compared to the pressures recorded at the end of treatment with clonidine alone ($p<0.01$) Since these changes were more significant than after propranolol + clonidine this suggests an extra effect of atenolol HR remained low Changing atenolol to propranolol again increased supine pressures and the differences now did not reach statistical significance when compared to clonidine alone

Addition of prazosin (1 mg t i d ) to the clonidine + propranolol treatment in 9 patients reduced BP ($p<0.05$) By contrast no effect was observed in standing BP Withdrawing propranolol from this combination elevated the pressures and HRs to the levels observed during treatment with clonidine alone The BPs and HRs at the end of the final phase on clonidine alone were comparable to those measured at the end of the first clonidine phase

*Side effects* of clonidine—drowsiness and dry mouth—were reported by 5 patients No new side effects were recorded during treatment periods when β blockers were added to clonidine During the clonidine + propranolol + prazosin period 3 out of these 5 patients had postural hypotension as a new side effect leading to their drop-out during this treatment phase

*In vitro results* As emerges from Fig 2 even high concentrations of clonidine alone produced only 50% of the maximum response of the tissue Propranolol and atenolol tended to enhance the clonidine responses Sotalol reduced clonidine induced contractions at low concentrations of clonidine without any clear effect at high clonidine concentrations Prazosin (10 ng/ml) entirely abolished clonidine responses When tested against noradrenaline induced contractions prazosin concentration dependently (0 1–10 ng/ml) shifted the noradrenaline dose–response graph to the right

## DISCUSSION

The design of the clinical study was open non randomized and fixed sequence with no placebo period at the end As such the design is modest Further the initial three week wash out from previous antihypertensive treatment with a diuretic can be too short However there is evidence (8) that the carry over effect of polythiazide (1 mg daily) enhanced the effect of prazosin at one week but no longer at two weeks after discontinuation of polythiazide Further since the BP measurements were actually blind and carried out by one and the same experienced nurse and since the effect of propranolol was documented twice and proved similar each time a non specific alteration of BP may not have been signficant

It has been warned that concurrent use of propranolol and clonidine should be avoided because hypertensive control may be lost (4) The evidence for this harmful interaction is rather scanty and is based largely on one report by Saarimaa (9) On the other hand numerous reports have confirmed the pressure raising effect of β-blockade during clonidine withdrawal reaction (2)

The present results show that combined administration of non selective β blocker propranolol and

clonidine does not increase the BP However the additive antihypertensive response of propranolol in this combination was less than the respective response to clonidine + atenolol (Table I) This could depend on the peripheral vasoconstriction and subsequent increase in total peripheral resistance due to the peripheral actions of both clonidine ($\alpha$ adrenoceptor agonist) and non selective $\beta$ blocker propranolol (blockade of vasodilator $\beta_2$-receptors) In these circumstances vasodilator prazosin proved useful

Clonidine is believed to act mainly on presynaptic $\alpha_2$-receptors which are probably scanty at postsynaptic sites—depending on animal species and the tissue concerned Bentley et al (3) have provided indirect evidence to suggest that $\alpha_2$-receptors do exist at postsynaptic sites in peripheral blood vessels Since their amount is probably low it is no wonder that isolated rabbit s aortic strips responded poorly to clonidine It is possible that clonidine in addition to its $\alpha$ sympathomimetic action also activates adenylate cyclase elimination by propranolol of this eventual effect should enhance clonidine responses Interestingly high concentrations of sotalol blocked the tissue responses to low concentrations of clonidine thus differing from two other $\beta$-blockers used This may be attributed to a non specific property of sotalol as a low concentration of it proved inactive (Fig 2) On the other hand sotalol did not antagonize the contraction due to high doses of clonidine and it is devoid of local anaesthetic properties (1) Thus an alternative interpretation is that sotalol has some extra effects on $\alpha_2$-receptors When applying this to practice a kind of clonidine withdrawal could result from such an interaction Further studies on this line are in progress using in situ preparation

The effect of low doses of prazosin added to propranolol + clonidine brought the supine BP to the levels otherwise recorded after atenolol + clonidine One can assume that prazosin eliminated the peripheral vasoconstrictor component of propranolol Published (6) and unpublished results suggest that prazosin added to $\beta$ blockers is able to reduce the high diastolic BP during isometric work which pressure otherwise is unresponsive to $\beta$-blockers

## ACKNOWLEDGEMENTS

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## REFERENCES


1 Avery G S (ed ) Cardiovascular drugs vol 2 $\beta$-Adrenoceptor blocking drugs Adis Press Hong Kong 1977
2 Bailey R R & Neale T J Rapid clonidine withdrawal with blood pressure overshoot exaggerated by beta-blockade Br Med J 1 942 1976
3 Bentley S M Drew G M & Whiting S B Evidence for two distinct types of postsynaptic $\alpha$ adrenoceptor Br J Pharmacol 61 116 1977
4 Bochner F Carruthers G Kampmann J & Steiner M Handbook of clinical pharmacology 1st ed p 112 Little Brown & Co Boston 1978
5 Furchgott R F Techniques for studying antagonism and potentiation of sympathomimetic drugs on isolated tissues In Animal and clinical pharmacologic techniques in drug evaluation vol 2 (ed P Siegler and J Moyer) pp 256–266 Year Book Medical Publishers Chicago 1967
6 Hunyor S & Nyberg G Comparison of intra arterial and indirect blood pressures at rest and during isometric exercise in hypertensive patients before and after metoprolol Br J Clin Pharmacol 6 (2) 109 1978
7 Kincaid Smith P Macdonald I M Hua A Laver M C & Fang P Changing concepts in the management of hypertension Med J Aust 1 327 1975
8 Kuokkanen K & Mattila, M J Demonstration of an additive antihypertensive effect of prazosin and polythiazide in outpatients Curr Ther Res 17 431 1975
9 Saarimaa H Combination of clonidine and sotalol in hypertension Br Med J 1 810 1976

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# Stokes-Adams Attacks Requiring Pacemaker Treatment in Three Patients with Acute Nonspecific Myocarditis

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ABSTRACT Three patients, aged 16–44 years with complete heart block in acute myocarditis are reported The diagnosis of myocarditis was based on the development of transitory repolarization disturbances on the ECG in association with clinical signs of acute infectious disease All patients were brought to hospital due to repeated Stokes-Adams attacks and demonstrated ventricular asystoles for up to 25 sec. The patients were all successfully treated with temporary intracardiac pacing but one of them later turned out to require a permanent pacemaker The possibility of differences in localization and in prognostic importance of conduction disturbances between infectious and ischemic myocardial disease is discussed

*Key words* acute myocarditis complete heart block pacemaker Stokes Adams attacks
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Acute myocarditis is generally considered as a complication with severe risks in various infectious diseases (1) This concept is mainly based on post mortem observations of infectious agents cell infiltration and myocardial cell degeneration in association with signs of acute heart failure (3 4) More recently corresponding studies of the conduction system of the heart have emphasized the importance of conduction defects and arrhythmias as the more immediate causes of sudden death in acute myocarditis (9) Moreover the improved techniques for supervising cardiac arrhythmias in recent years have substantially increased the possibilities to detect and consequently also to manage this type of complications to acute infectious myocarditis

However to date our knowledge of the arrhythmias that are responsible for sudden death in acute myocarditis is poor obviously due to their nexpected occurrence Only a limited number of es with Stokes Adams attacks due to proved complete heart block in acute myocarditis are reported in the literature (10) It might therefore be of interest to present three further cases admitted to our hospital during the last decade They also serve to complement our concept of an excellent acute and late prognosis in most cases of acute myocarditis as reported in an earlier follow up study (5)

## CASE REPORTS

*Case I*

A 44 year-old woman was admitted as an emergency case because of repeated short attacks of sudden unconsciousness developing after 3 weeks of sore throat and fever On the day before admission she had been prescribed penicillin because of an acute otitis media Similar attacks had occurred in 1959 during another persistent infection of the upper respiratory tract Apart from this her past history was uneventful

On admission the ECG demonstrated a prolonged P-Q interval with short periods of second degree AV block During the first 2 hours the patient developed at least 10 attacks of documented ventricular asystoles of up to 10 sec As the attacks continued in spite of isoprenaline infusion temporary pacemaker treatment was started by means of a transvenous intracardiac electrode

The patient had elevated body temperature (>38°C) during the first 3 days in hospital but otherwise remained asymptomatic The chest X-ray including heart volume was normal

*Laboratory investigations* on admission showed an ESR of 32 mm/hour a WBC of $9\times10^9$/l normal myocardial enzymes normal electrolytes and normal values of cholesterol and triglycerides Cultures from the nasopharynx showed growth of Staphylococcus aureus Tests for Coxsackie and echo viruses were negative and the AST ASTA titers normal

The pacemaker electrode was withdrawn after 2 days treatment when the atrioventricular conduction had normalized The QRS complex remained normal but there were transitory T wave changes in leads $CR_{2-4}$ (Fig 1)

In 1971 the patient was readmitted on account of identical attacks of unconsciousness 2 weeks after an upper respiratory infection The ECG again disclosed periods of ventricular standstill necessitating immediate pacemaker

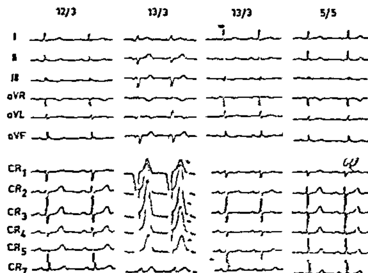



*Fig 1* ECG recordings from case 1 before during and after pacing with transitory local T wave changes in CR 2-4

treatment. The patient had a leucocytosis and an elevated ESR but bacterial cultures and serological studies showed negative results

The patient's manifest tendency to develop severe Stokes-Adams attacks in connection with innocent infections was considered to motivate permanent pacemaker treatment She has since demonstrated a normal exercise ECG and avoided further cardiac syncopes

*Case 2*

A 34-year-old mechanic was admitted because of a sudden syncope in his home He suffered from general malaise and fatigue but without obvious signs of infections for 3 days Shortly after admission during the physical examination the patient again lost consciousness with no auscultatory heart activity for 30 sec He was rapidly brought to the Intensive Care Unit where supervision ECG disclosed atrioventricular block of varying degree and repeated ventricular asystoles of up to 20 sec (Fig. 2) Pacemaker treatment by means of a transvenously inserted electrode was started During the following days he developed localized T wave changes in the inferior leads (Fig. 3) Atrioventricular conduction varied during the first days but after 10 days it was possible to discontinue pacemaker treatment in spite of a persistent first degree block

*Laboratory investigations* The patient had an elevated temperature (38°C) on admission and a high ESR (38 mm/h) Myocardial enzymes were within normal limits and so were other routinely examined laboratory values

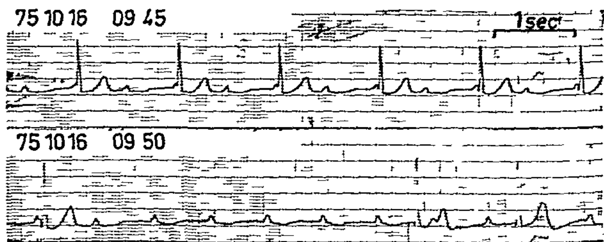
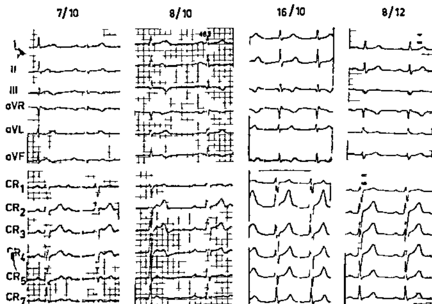



*Fig 2* ECG recording from case 2 during the stay in the Intensive Care Unit demonstrating AV block III with ventricular asystole of 4 sec



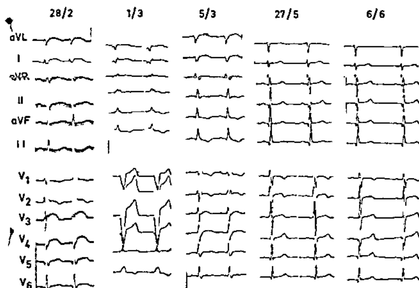

*F g 3* ECG record ngs from case 2 demonstrating a trans tory AV block III I and the successıve de elopment and later dısappearance of T wa e changes

Se ological stud es of ant bod es aga nst nfluenza ade o rus resp ratory syncytıal v rus mycoplasma echo v rus and Coxsack e were negat ve

Chest X ray d sclosed a sl ght enlargement of the heart (550 ml/m ) Vector ECG ga e no ev dence of myocardial nfarct on and there were no s gns of coronary ınsuff c en cy at max mal exerc se test ? mon hs after d scharge The f st degree block and the T wa e changes had d sap peared The pa ent thereafter returned to h s heavy wo k w thout further card ac symptoms

*Case 3*

A 16-year old schoolboy was adm tted to the Depart ment of Infect ous Dıseases due to syncopal attacks w th convuls ons after a few days of h gh fever (39–40°C) headache and gastro ntest nal symptoms Phys cal exam nat on on adm ss on revealed a fully consc ous boy w th out neck stiffness or other neurological s gns but w th an rregular and somet mes slow pulse Durıng h s first hour n hosp tal the pat ent developed several Stokes Adams attacks the ECG demonstrat ng a b fasc cular block




*F g 4* ECG record ngs from case 3 demonstrat ng a trans tory b fascıcular block temporary pac ng and the success e decrease ın n t al T wave changes o er the an terıor myocard al wall

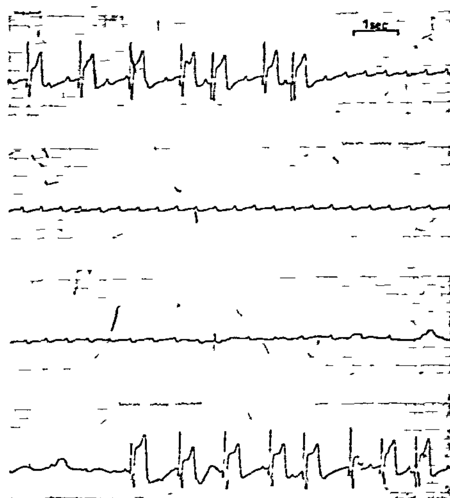



*Fig 5* Recording of a 25-second period of ventricular asystole in case 3

localized marked T wave changes over the anterior myocardial wall (Fig 4) and alternating degrees of AV block with repeated periods of ventricular asystole for up to 25 sec (Fig 5) Infusion of isoprenaline was instituted but as the attacks continued pacemaker treatment was started

During the following days the patient had moderate fever but no other symptoms The chest X ray demonstrated a slightly enlarged heart (560 ml/m²)

*Laboratory investigations* on admission showed an ESR of 30 mm/hour and a WBC of 10×10⁹/l The S ASAT values were elevated during the first week with a maximum of 3 4 μkat/l Serum electrophoresis showed an inflammatory reaction with raised IgM Cultures from blood nasopharynx and urine were negative and so were tissue cultures from faeces and throat swabs Serological examinations showed no significant titers of antibodies against influenza adenovirus mycoplasma ornithosis echo virus or Coxsackie

Five days after admission a noventricular conduction was restituted but the ECG disclosed a remaining right bundle branch block in combination with a left posterior hemiblock The patient s condition improved rapidly and he could leave the hospital after 3 weeks The intraventricular conduction disturbances did not disappear until 3 months after discharge The T wave changes partly remained for another 2 months (Fig 4)

## DISCUSSION

The diagnosis of acute myocarditis in the present study was based on the appearance of transitory T wave changes in the ECG and Stokes-Adams attacks due to proved ventricular standstill in patients with clinical signs of acute infectious disease but without evidence of coronary insufficiency On the other hand identifying the responsible agent is an obvious problem in view of the well known difficulties of sampling and culturing representative microorganisms and the short-coming of serological techniques particularly in infections of viral origin

The low incidence of complete AV block in acute

myocarditis has been attributed to the observation that this disease irrespective of the responsible agent mainly involves the distal parts of the con duction system (9) Coronary insufficiency has more obvious possibilities to affect proximal con ducting structures notably the AV node explaining the more frequent occurrence of AV blocks in acute myocardial infarction

At the same time this difference in preferential level of conduction defects between infectious and ischaemic myocardial disease may have prognostic implications The peripheral involvement of the conduction system in acute myocarditis should thus be expected to favour the development of ventricu lar asystole due to the risk of multifascicular affec tion provided that the myocardial lesion is suffi ciently extensive This hypothesis is supported by the study of Lim et al (10) who reported trifascicu lar block in 8 out of 10 patients with Stokes Adams attacks due to acute nonspecific myocarditis Simi larly our patient with the most severe asystoles (case 3) had shown bifascicular block and extensive T wave changes Similar repolarization disturb- ances were usually found also in the study by Lim et al and suggest extensive myocardial involve ment as a prerequisite for this type of severe con duction defects The frequent combination with elevated ESR values and myocardial serum enzymes is an indication in the same direction as is the fact that 3 of the 10 patients studied by Lim et al (10) remained in cardiogenic shock even when paced

On the other hand it is possible that the conduc tion system is sometimes affected more selectively in acute myocarditis In case 1 the T wave changes were localized and transitory but the attacks of ventricular standstill nevertheless dramatic

The restitution of conduction after asystoles due to acute myocarditis is usually reported to occur within a few days allowing the situation to be man aged by temporary pacing (6 7 10) or sometimes by steroids (8) Some patients however require per manent pacing (2 11) Our case 1 indicates that such a demand may not be established until several years after the initial episode This type of late relapse of Stokes Adams attacks after previous myocarditis stresses the need for future supervision of such patients in the event of later infection Our cases 2 and 3 illustrate the value of access to a qualified emergency service in cases of infectious diseases with initial fainting symptoms

## REFERENCES


1 Abelmann W H Viral myocarditis and its sequelae Ann Rev Med 24 145 1973
2 Bairan A C Cherry J D Fagan L F & Codd J E Jr Complete heart block and respiratory syncy tial virus Am J Dis Child 127 264 1974
3 Bell R W & Murphy W M Myocarditis in young military personnel Am Heart J 74 309 1967
4 Burch G E Sun S C Colcolough H L Sohal R S & De Pasquale N P Coxackie B viral myocar ditis and valvulitis identified in routine autopsy specimens by immunofluorescent techniques Am Heart J 74 13 1967
5 Gerzen P Granath A Holmgren B & Zetterquist S Acute myocarditis A follow up study Br Heart J 6 575 1972
6 Giles T D & Gohd R S Respiratory syncytial virus and heart disease JAMA 236 1128 1976
7 Johnson J L & Lee L P Complete atrioventricu lar heart block secondary to acute myocarditis requir ing intracardiac pacing J Pediatr 78 312 1971
8 Kirmser R Umbach R Rowett D & Ross A Complete heart block due to acute nonspecific car ditis Chest 71 682 1977
9 Lev M & Bharati S Atrioventricular and intraven tricular conduction disease Arch Intern Med 135 405 1975
10 Lim C H Toh C C S Chia B L & Low L P Stokes Adams attacks due to acute nonspecific myocarditis Am Heart J 90 172 1975
11 Schieken R M & Myers M G Complete heart block in viral myocarditis J Pediatr 87 831 1975

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# Enteric Coated Quinidine Compared to Sustained Release Preparations during Repeated Administration

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ABSTRACT The concentration of quinidine in plasma was measured in 12 healthy subjects during multiple administration of an enteric coated tablet (Systodin®) and two sustained release preparations (Kinidin Duretter® and Kinilentin®) In a second study, involving another 12 subjects, the enteric coated tablet and the most widely used sustained release preparation (Kinidin Duretter®) were compared with plain uncoated quinidine sulphate tablets in order to calculate the relative bioavailability of the formulations used for maintenance therapy The largest area under the plasma concentration time curve ($AUC_{12h}$) during a dosage interval (12 hours) was obtained with the plain tablets and with the enteric coated formulation The variation of the plasma concentrations during the dosage interval was not larger with the enteric coated tablets than with the sustained release preparations The time of appearance of peak concentration after administration was longer and more variable with the enteric coated tablets In relation to the plain quinidine tablets, the bioavailability of Systodin and Kinidin Duretter was 96% and 84%, respectively In 21 out of 24 cross-over experiments with Kinidin Duretter and Systodin the $AUC_{12h}$ was larger with the latter formulation Enteric coating appears to be a simple and reliable means of achieving delayed absorption and stable quinidine plasma levels during maintenance therapy

*Key words* quinidine biological availability
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Sustained release quinidine preparations producing relatively stable plasma concentrations are routinely used for maintenance therapy in patients with cardiac arrythmias A number of proprietary sustained release formulations and salts with delayed absorption are available permitting quinidine to be given orally at 12 hour intervals The most thoroughly studied preparation is Kinidin Duretter® (Quinidine Durules®) which has a large share of the market in many parts of the world This formulation is well absorbed and the variation in the plasma concentration of quinidine during the dosage interval is within acceptable limits (2 4 8)

Enteric coating represents a less sophisticated manufacturing principle which is not subject to patent protection According to recent WHO recommendations quinidine is an essential drug for which adequate supplies and manufacturing facilities should be available also in the Third World (12) It was therefore deemed of interest to compare the plasma concentration time profile and the bioavailability of an enteric coated preparation (Systodin®) and of two sustained release dosage forms (Kinidin Duretter® and Kinilentin®) during multiple dosing under carefully controlled conditions

## MATERIAL AND METHODS

*Human subjects*

All candidates underwent a physical examination supplemented with a 12 lead ECG a complete haematological status and laboratory tests for liver and renal function Only subjects with normal findings were selected for the study The volunteers were informed about the purpose of the experiments and the possible adverse effects of quinidine treatment and gave their written consent to participate Before the study was initiated a test dose of quinidine sulphate (0 4 g) was given and the ECG was repeated after 2 hours

*Quinidine preparations*

Four different formulations were studied (*A*) Enteric coated tablets containing 0 2 g of quinidine sulphate (Sys

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*Abbreviations* $AUC_{12h}$ = area under the plasma concentration time curve during the 12 hour dosage interval $C_{max}$ = highest concentration of quinidine $C_{min}$ = lowest concentration of quinidine $t_m$ = time of appearance of the highest concentration ANOVA = analysis of variance CAP = cellulose acetate phthalate

Table I Q d e co tent (°o of label cl ) f t e prep rat o s st d ed ( iea i + S D )

| Prep ara t on | Batch no | No of doses analysed | Qu n d ne content |
|---|---|---|---|
| *A* | 704018<br>608166 | 10 | 100 1+4 6 |
| *B* | 830201 DA 467<br>811 01 BL 431 | 10 | 103 1+7 7 |
| C | B 38 M<br>B 40 D | 10 | 101 3+4 8 |
| *D* | 610619 | 10 | 107 8+4 8 |

ª The mean of repeated ($n$=8) analyses of authent c qu n d ne sulpha e (400 mg) taken as 100%

tod n® A/S Farmaceut sk Industri Oslo Norway) The cellulo e acetate phthalate (CAP) coated tablets were n tact after 2 hours n s mulated gastric ju ce and the d sso-lut on t me ($t_{50}$ ) n pH 6 8 phospha e buffer was ap p oximately 11 12 m n (personal commun cat on f om the manufacturer) (*B*) Sustained release tablets conta n ng qu n d ne b sulphate equ alent to 0 2 g of the sulphate embedded n an nsoluble porous matrix of plast c res n (K n d n Duretter® Qu n d ne Durules® Hassle Gothen burg Sweden) The d ssolut on characterist cs of th s preparat on have been reported elsewhere (9) (*C*) Sus tained release sugar coated tablets contain ng qu n d ne b sulphate equ valent to 0 2 of the suphate embedded n a matrix of a fat alcohol (K n lent n® Leo Laborator es Ballerup Denma k) (*D*) Uncoated pla n tablets conta n ng 0 1 g of qu n d ne sulphate (NAF Laboratories Oslo Norway)

The qu n d ne content of the preparat ons was de er m ned as described by Regårdh et al (9 Representat ve samples were obta ned by analys ng 5 doses f om each of the 2 batches of p eparat ons *A* *B* and *C* and 10 doses of the pla n tablets *D* (Table I)

*Expe en al proto ol*

Twel e of the volunteers (6 males and 6 females) aged 22–40 years (mean 31) and we gh ng 55 74 kg (mean 65 6) part c pated in the f rst series of experiments (study 1) w th preparat ons *A* *B* and *C* A cross o er des gn was used so that all of the subje s ece ed the three p epara t ons Each of the s x poss b e p epa at on sequences was ass gned to two subjects acco d ng o a des gn ncorporat ng 4 repl cate 3×3 Lat n squares 11

The dose was 0 4 g (2 table e e y 12 hours and the volunteers were nstructed to take he morn ng dose w th the r usual breakfast The f rst p epa a on n the sequence was taken for 4 days and the fo ow ng two for 2 days each Blood samples (heparin zed were aken at 2 4 6 8 and 12 hours after the morn ng d e n the last dosage nterval w th each preparat on Add t onal blood samples were drawn from 5 of the subjects 48 hours after the last dose in order to test the protocol fo arry over effects

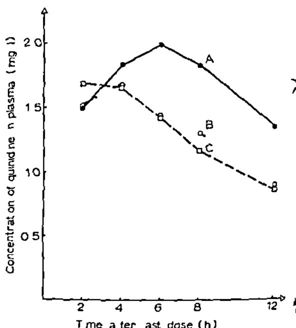
Table II C ce tr t i f l l c pl s t l ff re t t es d r ig l s ge ter l (12 l) il f r or l prep r t o s

| Hours after last dose | Concentra on of qu n d ne (mg/l)ª *A* | *B* | *C/D* |
|---|---|---|---|
| *St dy 1 (A B a d C)* | | | |
| 2 | 1 50+0 40 | 1 52+0 42 | 1 69+0 30 |
| 4 | 1 84+0 77 | 1 69+0 42 | 1 67+0 40 |
| 6 | 2 01+0 64 | 1 45+0 32 | 1 44+0 21 |
| 8 | 1 84+0 49 | 1 31+0 22 | 1 18+0 24 |
| 12 | 1 36+0 76 | 0 90±0 19 | 0 86+0 19 |
| *St d 2 (A B and D)* | | | |
| 2 | 1 52+0 46 | 1 45 0 34 | 2 20+0 73 |
| 4 | 1 68+0 68 | 1 49+0 27 | 1 87+0 55 |
| 6 | 1 53+0 52 | 1 36+0 25 | 1 61+0 59 |
| 8 | 1 38±0 42 | 1 20+0 27 | 1 25+0 4 |
| 12 | 1 15+0 31 | 0 94+0 23 | 0 89+0 4 |

ª Mean + S D of 12 observat ons n each study corrected for dev at ons from label cla m to 400 mg dose every 12 h

The plasma was stored at 20°C un l analysed A com plete haematolog cal status was repeated af er the experi ments

Study 2 nvolved 12 subjects (11 males and 1 female) aged 22–41 years (mean 30) and we gh ng 64–84 kg (mean 71 7) They were g ven p eparat ons *A* *B* and *D* The protocol was ident cal to that of study 1 and the dose was 0 4 g (2 tablets of p eparat ons *A* and *B* 4 tablets of preparat on *D*) every 12 hours




*F g 1* Concentrat on of qu n d ne n plasma during a dosage nterval w th preparat ons *A* *B* and *C*

Table III *Bioavailability parameters corrected for deviations from label claim to 400 mg dose every 12 h (mean ± S D)*

| | Preparation | | | ANOVA | Tukey's test ($p$) | | |
|---|---|---|---|---|---|---|---|
| | *A* | *B* | *C/D* | ($p$) | *A B* | *A C/D* | *B C/D* |
| *Study 1 (A, B and C)* | | | | | | | |
| $AUC_{12h}$ (mg h/l) | 20.3 ± 5.1 | 15.9 ± 3.1 | 15.7 ± 2.8 | 0.007 | <0.025* | <0.025* | NS |
| $C_{max}$ (mg/l) | 2.15 ± 0.64 | 1.74 ± 0.38 | 1.81 ± 0.35 | 0.06 NS | | | |
| $C_{max}/C_{min}$ | 1.75 ± 0.41 | 1.94 ± 0.30 | 2.18 ± 0.57 | 0.07 NS | | | |
| $t_{max}$ (h) | 6.0 ± 2.6 | 3.7 ± 1.1 | 2.8 ± 1.0 | 0.000 | <0.005* | <0.001* | NS |
| *Study 2 (A, B and D)* | | | | | | | |
| $AUC_{12h}$ (mg h/l) | 17.0 ± 4.7 | 15.0 ± 3.0 | 17.8 ± 6.0 | 0.06 NS | | | |
| $C_{max}$ (mg/l) | 1.87 ± 0.54 | 1.54 ± 0.30 | 2.70 ± 0.73 | 0.0009* | NS | NS | <0.001* |
| $C_{max}/C_{min}$ | 1.81 ± 0.37 | 1.69 ± 0.31 | 2.59 ± 0.44 | <0.0001* | NS | <0.001* | <0.001* |
| $t_{max}$ (h) | 4.1 ± 2.0 | 3.7 ± 1.1 | 2.1 ± 0.3 | 0.0007* | NS | <0.001* | <0.01* |

* $p<0.05$ NS = not significant

*Analysis and calculations*

The concentration of quinidine in plasma was determined using the spectrophotofluorimetric method of Cramer and Isaksson (1). The individual plasma concentrations were corrected for deviations from claimed content of drug (Table I) to dosage 400 mg every 12 hours.

The area under the plasma concentration time curve during one dosage interval ($AUC_{12h}$) was calculated by the trapezoidal rule assuming that the concentrations immediately before the morning and evening doses were equal. The ratio between the highest concentration ($C_{max}$) and the lowest concentration ($C_{min}$) in the interval was calculated for each of the drugs. The time of the appearance of the highest concentration ($t_{max}$) was recorded or in cases with two equal peak concentrations calculated as the mean of the corresponding times.

Studies 1 and 2 were subjected to separate statistical analyses. Analysis of variance for cross-over design was carried out with the four derived parameters assuming additivity and no interactions between the treatments, the subjects and the preparation sequence. Where statistically significant differences ($p<0.05$) were observed in the analysis of variance (ANOVA) the three preparations were compared using Tukey's a posteriori test as recommended by Wagner (11).

## RESULTS

*Analysis of the formulations*

The quinidine contents of the preparations are shown in Table I. The largest deviation from the claimed content (7.8%) was found with the plain uncoated tablets (*D*) included in the study as a reference preparation for the calculation of relative bioavailability of the three formulations used for maintenance therapy. Since the drug contents of preparations *A*, *B* and *C* were close to the declared amounts only small corrections were required for their observed plasma concentrations.

*Study 1*

At all times except 2 hours after the morning dose the highest mean concentration of quinidine was found with the enteric coated tablets (*A*). The peak mean concentration occurred at 6 hours after the dose (Table II, Fig. 1). There was little difference between the two sustained release preparations (*B* and *C*) with regard to their plasma concentration time profiles.

$AUC_{12h}$ was significantly larger ($p<0.025$) with preparation *A* than with preparations *B* and *C* (Table III). Interestingly the lowest mean $C_{max}/C_{min}$ ratio (1.75) was found with preparation *A*. However the differences between the preparations with regard to this variable were not statistically significant. On the other hand $t_{max}$ was significantly longer with preparation *A* than with preparations *B* ($p<0.005$) and *C* ($p<0.001$). The interindividual differences were also more pronounced with preparation *A*.

The concentration of quinidine in plasma at 48 hours after the last dose of the 8-day treatment period was only 0.1–0.2 mg/l.

*Study 2*

Again the mean plasma concentrations of quinidine were higher with preparation *A* than with *B* (Table

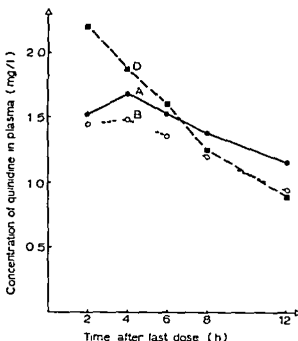



*Fig 2* Concentration of quinidine in plasma during a dosage interval with preparations *A* *B* and *D*

II Fig 2) The concentration at 2 hours after the dose was considerably higher with preparation *D*

The differences in $AUC_{12\ h}$ during the treatment periods (Table III) did not reach statistical significance ($p=0\ 06$) Using the area for tablets *D* as a reference (100%) the calculated bioavailability of preparations *A* and *B* was 96% and 84% respectively As expected $C_{max}$ and the ratio $C_{max}/C_{min}$ were significantly higher ($p<0\ 001$) with preparation *D* than with *A* and *B* The ratio $C_{max}/C_{min}$ was similar with the latter two preparations

Mean $t_{max}$ was only marginally longer with *A* tablets but the interindividual variation was more pronounced The shortest $t_{max}$ was found with preparation *D*

## DISCUSSION

In view of the potential haematological side effects of quinidine the minimal recommended doses were used and the treatment periods were as short as possible However steady state plasma concentrations of quinidine are attained when the drug is given at 12 hour intervals for 48 hours (4) Therefore the present experimental design administering the first formulation for 4 days before blood sampling leaves little doubt that the quinidine concentrations were measured at steady state The treatment period with each of the subsequent preparations (48 hours) was approximately 6–7 fold the mean elimination half life of quinidine (3 9) Nevertheless the observed drug concentrations at 48 hours after the last dose suggest that there is a small (<10–20%) carry over effect in the protocol The most likely explanation for this observation is delayed absorption of some of the formulations

Since the first published reports on quinidine concentrations in blood after single and multiple doses of preparation *A* (6 7) the thickness of the CAP layer of the cotaing has been reduced (personal communication from the manufacturer) However, higher peak concentrations and longer $t_{max}$ with the enteric coated *A* tablets compared to the sustained release preparation *B* were also found in these earlier studies

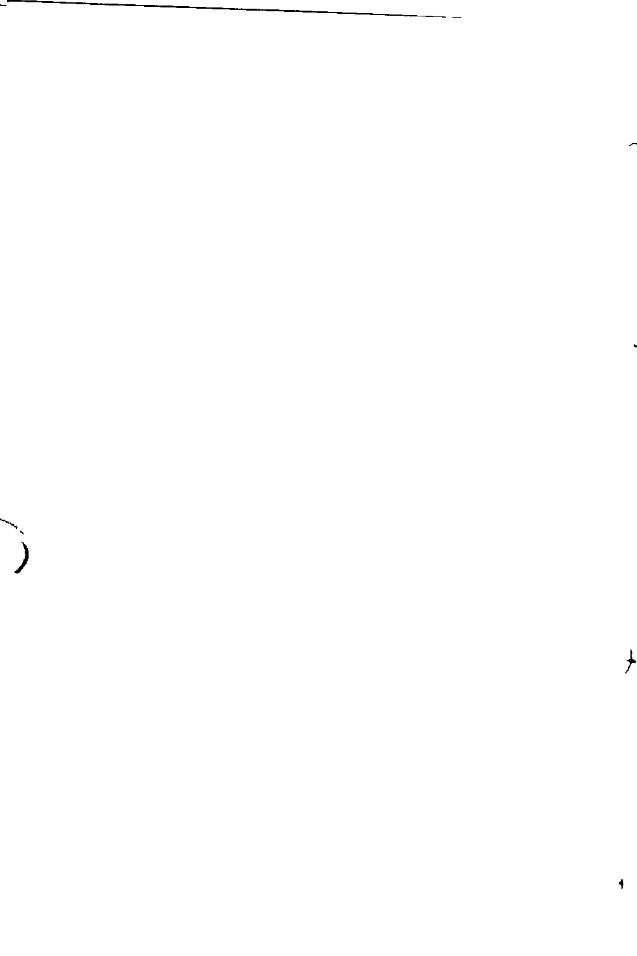
The plasma concentrations observed with preparations *A* and *B* were somewhat lower in study 2 than in study 1 This is probably related to the higher mean body weight (approximately 10%) of the subjects who volunteered for the second series of experiments

Preparation *A* produced larger $AUC_{12h}$ than preparation *B* in 21 of the 24 subjects who took both formulations The calculated bioavailability of 96% for preparation *A* relative to preparation *D* suggests that the enteric coating does not interfere with the total absorption under the experimental conditions of the present study The relative bioavailability observed with preparation *B* (84%) is in good agreement with the findings of Henning and Nyberg (4) and Mahon et al (8) who reported values of 77% and 90% respectively

A recent study of three sustained release quinidine tablets showed that the dose corrected AUC was similar whereas their plasma concentration time profiles were different (5) The present finding of satisfactory bioavailability with an enteric coated preparation compared to the more sophisticated sustained release tablets is therefore noteworthy Since the $C_{max}/C_{min}$ ratio is not higher with the enteric coated tablets when the drug is given for maintenance therapy a moderate delay in the appearance of peak concentrations is not important It appears that enteric coating represents a feasible alternative to the more sophisticated sustained release principle which has gained acceptance as a method of achieving stable quinidine concentrations in the blood with 12 hour dose intervals

## REFERENCES

1 Cramer G & Isaksson B Quantitative determination of quinidine in plasma Scand J Clin Lab Invest 15 553 1963
2 Frigo G M Perucca E Teggia Droghi M Gatti G Mussini A & Salerno J Comparison of quinidine plasma concentration curves following oral administration of some short and long acting formulations Br J Clin Pharmacol 4 449 1977
3 Greenblatt D J Pfeifer H J Ochs H R Franke L MacLaughlin D S Smith T W & Koch Weser J Pharmacokinetics of quinidine in humans after intravenous intramuscular and oral administration J Pharmacol Exp Ther 202 365 1977
4 Henning R & Nyberg G Serum quinidine levels after administration of three different quinidine preparations Eur J Clin Pharmacol 6 239 1973
5 Huynh Ngoc T Chabot M & Siros G Bioavailability of three commercial sustained release tablets of quinidine in maintenance therapy J Pharm Sci 67 1456 1978
6 Lindseth Ditlefsen E M Concentrations of quinidine in blood following delayed absorption tablets Acta Med Scand 149 49 1954
7 Lindseth Ditlefsen E M & Løken H F Quinidine concentrations in serum following two different types of delayed absorption tablets Acta Med Scand 179 333 1966
8 Mahon W A Mayersohn M & Inaba T Disposition kinetics of two oral forms of quinidine Clin Pharmacol Ther 19 566 1976
9 Regårdh C G Johnsson G Lundborg P & Persson B A Bioavailability of quinidine in slow release form Arzneim Forsch 27 1 1977
10 Ueda C T Hirschfeld D S Scheinman M M Rowland M Williamson B J & Dzindzio B S Disposition kinetics of quinidine Clin Pharmacol Ther 19 30 1976
11 Wagner J G Fundamentals of clinical pharmacokinetics 1st ed Drug Intelligence Publications Hamilton 1975
12 WHO Expert Committee The selection of essential drugs WHO Tech Rep Ser 615 1975



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# Does Aortocoronary Saphenous Vein Bypass Surgery Change the Native Coronary Arteries?

*An Angiographic Follow up of 60 Patients*

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ABSTRACT Progression of disease in the native coronary circulation was studied in 60 patients before and after aortocoronary saphenous vein bypass surgery The mean interval between the first angiographic study and operation was 2 0 months and the mean interval from operation to second catheteriza tion was 15.3 months The results from a second arteriographic study after an interval of 12.3 months on 25 comparable patients with no surgery in be tween, served as a control material for frequency and severity of the 'natural' progression of the disease Only vessels which were not occluded at the first examination were included in the re study In the 60 operated patients, 106 arteries were grafted and 74 ungrafted Total graft patency at the time of re study was 66% Progression was found in segments proxi mal to the anastomosis in 42% of arteries with patent graft, and in 47% of arteries with occluded graft whereas in segments distal to the site of anastomosis progression was found in 5% of the arteries with patent graft and in 55% of arteries with occluded graft Progression of the disease was found in 11% of ungrafted arteries In the control group, progression of the disease was found in 24% The study thus shows that after aortocoronary bypass surgery progression in segments proximal to the anastomosis is significantly higher than in ungrafted arteries The progression was independent of patency or occlu sion of the graf to the vessel Progression in the seg ments distal to the anastomosis was not significantly different from the frequency of progression seen in nongrafted arteries if the graft was patent, but was much higher if the graft was occluded

*Key words* aortocoronary saphenous vein bypass coronary artery disease progression

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Myocardial revascularization by interposition of a saphenous vein graft between the aorta and the distal portion of an obstructed coronary artery is an attractive alternative in the management of patients with angina pectoris Although the results have been encouraging in the majority of patients many important questions need to be answered before the final evaluation of this treatment is completed One critical point is the effect of the bypass on the native coronary artery circulation Some reports have suggested that obstructive lesions may progress more rapidly in coronary arteries to which venous bypass grafts have been attached than would be expected from the natural course of the disease (1 5 9 12) while other investigators have been unable to demonstrate any relation between surgery and progression of the disease (2 7)

We report the observed changes in grafted and ungrafted coronary arteries in 60 patients who had a second angiography an average of 15 3 months after bypass surgery For comparison data are presented from a similar re study of 25 patients with coronary artery disease with no surgical intervention

## PATIENTS AND METHODS

Pre and postoperative coronary arteriograms were obtained from 60 consecutive patients (56 men and 4 women) aged 35–65 years who underwent aortocoronary saphenous vein bypass surgery to right circumflex or left descending coronary artery at this hospital in 1974–76 The indication for bypass was stable angina pectoris which medical treatment had failed to relieve Pre operatively all patients had class III–IV (NYHA) symptomatology and all were improved by at least one class at the time of re study None of the patients were on anticoagulant therapy All operations were performed with total cardiopulmonary bypass hemodilution moderate hypothermia ventricular fibrillation and left ventricular decompression Only vessels with adequate distal run-off on preoperative angiography (lumen diameter >1–2 mm) and without significant distal obstruction were considered suitable for grafting The veins for use as grafts were taken from the greater saphenous system between

Table I *Distribution of score at initial examination*

| Score | Controls No of arteries | % | Operated patients No of arteries | % |
|---|---|---|---|---|
| 0 | 13 | 17 | 8 | 4 |
| 1 | 2 | 3 | 17 | 9 |
| 2 | 13 | 17 | 13 | 8 |
| 3 | 9 | 12 | 41 | 23 |
| 4 | 18 | 24 | 49 | 27 |
| 5 | 20 | 27 | 52 | 29 |
| Total | 75 | 100 | 180 | 100 |

the groin and knee in all patients Most anastomoses to coronary arteries were performed with periods of aortic occlusion and with tapes used only occasionally Mechanical arterial dilation was used sparingly and concomitant endarterectomy was not performed The mean interval between the first examination and surgery was 2 0 months The mean interval from operation to second catheterization was 15 3 months (range 7–36)

Twenty five patients (22 men and 3 women) of comparable age who had a second catheterization after an interval of 12 3 months (range 7–16) with no surgery in between served as a control group for frequency and severity of the natural progression of the disease For various reasons bypass surgery in these patients had been postponed for more than 3 months after the first angiography necessitating a new preoperative examination

The first angiographic study showed that the majority of patients had two or all three of their main coronary arteries affected so that single vessel disease was uncommon In this respect there was no difference between the findings in the operated patients and the controls (Table I)

*Selective coronary arteriography* was performed by the percutaneous transfemoral method of Judkins (10) The contrast material used was Isopaque® Coronar (metrizoate meglumine/Na/Ca (58/9/1) 370 mg iodine/ml) Preshaped catheters (Ducor Cordis Corporation) were used and hand injections of 3–9 ml contrast medium were given repeatedly Cinerecordings were performed with a 35 mm Arriflex camera combined with an image intensifier running 75 frames/sec The coronary arteriograms were performed in the left anterior and right anterior oblique position and additional projections were used when needed The three main coronary arteries—the anterior descending branch and the circumflex branch of the left coronary artery and the right coronary branches—were identified and evaluated separately The obstructions were scored on a percentage basis 0 = No arteriographic abnormalities seen 1 = Trivial irregularity(ies) estimated to be less than 50% of the luminal cross sectional area 2 = Localized narrowing estimated to be at least 50% but less than 75% of the luminal cross sectional area 3 = Narrowing(s) estimated to be at least 75% but less than 90% of the luminal cross sectional area 4 = Narrowing(s) estimated to be more than 90% of the luminal cross sectional area 5 = Total obstruction of a vessel Adding the score values and dividing by the number of arteries examined gave a mean artery score Grading of per cent obstruction was easily performed for nongrafted arteries and arteries with patent grafts With occluded grafts the segment of the native vessel proximal to the anastomosis could be visualized by injection into the vessel but if the proximal lesion had progressed to total occlusion the section of the artery distal to the anastomosis could be analyzed only if it was filled through collateral vessels The grafts were generally injected selectively or in some cases visualized after an aortic root injection

The radiographic and processing techniques were constant throughout the study The arteriograms were analysed by a single observer (K L ) who was not aware of whose arteriograms he was studying Progression was considered to have occurred in a segment in which the degree of narrowing had increased by at least one score Only vessels which were not occluded at the preoperative examination were included in the re study

Arithmetical means and standard deviations were calculated as described by Snedecor (19) The statistical difference between means was evaluated with Student s $t$ test $P$ values above 0 05 were not considered significant

## RESULTS

### *Control group*

Of a total of 75 main arteries in the control series 20 were totally occluded at the first study and are not further discussed The mean score in the remaining 55 arteries increased from 2 3±1 5 to 2 6±1 6 which was not significant ($t$=1 100 $p$<0 05) Forty two arteries showed no significant progression while 13 arteries revealed progression which amounted to total occlusion in 3 Thus the total progression rate that is the sum of artery progression to total occlusion among arteries showing an increase in percentage stenosis was 13/55 (24%) Arteries with moderate or severe disease had the greatest chance of developing new total occlusion or progressive stenosis The changes were of similar magnitude when the left anterior descending artery the right coronary artery and the circumflex artery were examined separately

### *Grafted arteries*

*Progression proximal to the anastomosis* Of 106 grafted arteries 33 with a completely occluded proximal segment in the preoperative angiogram are not discussed further Of the remaining 73 arteries 45 had a patent graft while 28 had an occluded graft at the re study In the former the mean arterial score in segments proximal to the site of anastomosis increased from 3 4±0 7 to 3 8±1 1 ($t$=2 368 0 05>$p$>0 01) 26 proximal segments

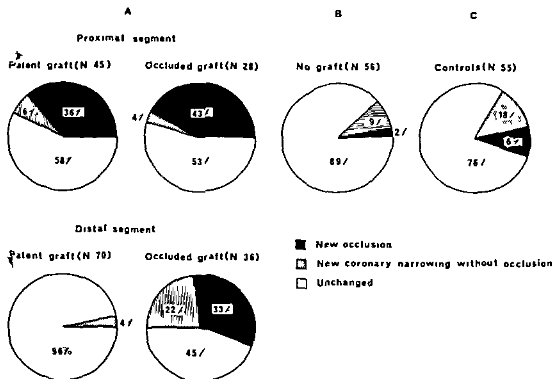



*Fig 1* Incidence of new occlusions new coronary narrowing without occlusion or lack of changes in (*A*) grafted arteries with patent or occluded vein grafts (*B*) ungrafted arteries in operated patients and (*C*) arteries in unoperated patients (control group) In parentheses number of arteries restudied

were unchanged and 19 segments showed progression which amounted to total occlusion in 16 Thus progression of the disease proximal to the anastomosis occurred in 19/45 (42%) The mean arterial score in segments proximal to the site of anastomosis in 28 vessels with occluded graft increased from 3 6±0 6 to 4 2±0 8 ($t$=2 928 0 05>$p$>0 01) In 15 arteries segments proximal to the anastomosis showed no progression while 13 arteries showed increased proximal stenosis which amounted to total occlusion in 12 Thus the progression rate for this group was 13/28 (47%)

The progression of the disease was most pronounced in arteries with severe proximal stenosis before operation Twenty three (58%) of the proximal segments with 90% stenosis in preoperative angiograms were found to be occluded at the time of re study and 5/27 (19%) of segments with moderate disease (75–90%) while new occlusion or progression without occlusion was not seen in segments with less severe disease (less than 75% stenosis)

*Progression distal to the anastomosis* Before operation the segment of the parent artery distal to the site of graft anastomosis was open or showed minimal disease (<50%) The mean arterial score in the distal segment in 70 arteries with patent graft was 0 5±1 1 before surgery and 0 3±0 8 at the re study the small difference being insignificant ($t$=0 780 $p$>0 05) Sixty seven segments showed no progression while increased percentage stenosis was observed in 3 segments although none of them were occluded Thus the progression rate for the distal segment when the graft was patent was 3/70 (4%) The mean arterial score in the distal segment of 36 arteries with occluded graft increased from 0 5±0 6 to 2 3±2 1 ($t$=4 88 $p$<0 001) Sixteen segments showed no progression of the disease while a significant increase in percentage stenosis was observed in 20 segments amounting to total occlusion in 12 Thus the progression rate for the distal segment of the parent artery when the graft was occluded was 20/36 (55%)

*Progression in nongrafted arteries* In the 60 patients with bypass surgery there were 74 ungrafted main arteries Of these a total of 19 were occl

in the preoperative angiogram The average score in the remaining 55 nongrafted arteries increased in significantly from 2 0±1 3 to 2 2±1 4 ($t$=0 683 $p$>0 05) Fifty arteries showed no progression of the disease in the second study while the lesion progressed in 6 to total occlusion in one and to subtotal in 5 Thus the total progression rate for this group was 6/56 (11%)

*Comparison between grafted and nongrafted arteries* Fig 1 summarizes the changes after surgery in the proximal and distal segment of the parent arteries in the same way as above The results from studies of nongrafted arteries in the operated and in the control group are also presented New and progressive proximal lesions were encountered at about the same frequency in arteries with patent as with occluded graft As far as the distal segment of the native artery is concerned the rate of progression was much higher in arteries with occluded grafts New total occlusions new obstructive lesions and progression of pre existing lesions were much more frequent in grafted than in nongrafted arteries in the operated group and also more frequent than in the control group The conclusions drawn from this are however limited by the fact that the initial diseases in the various subgroups were not strictly comparable

We therefore also analysed the precursors of an occlusion that developed in ungrafted or grafted arteries (proximal segment) that is how often an occlusion developed from a nearly obliterated lumen (90–99%) or from a lesser degree of narrowing It was quite apparent that the majority of occlusions occurred in arteries which were already obstructed to 90–99% but that this phenomenon occurred more frequently in grafted arteries (57%) than in nongrafted (22%)

## DISCUSSION

The aim of this study was to describe the changes in coronary angiograms in patients with coronary artery disease after saphenous vein bypass surgery Being well aware of the limitations of the present method (3) considerable care was taken to ensure that the sets of films were comparable particulary with regard to magnification and multiple views of the coronary arteries were taken in different projections All injections were made by hand Continuous pressure recordings were made in order to avoid the potential hazards of wedge coronary or graft injections (18) All our examinations were carried out in the same laboratory using the same type of catheter and contrast medium

Postoperative angiographic studies have yielded conflicting results regarding progression of the disease in bypassed coronary arteries Some investigators have been unable to demonstrate any relation between surgery and progression of the disease (2 7) some have documented an increased incidence of progression in vessels with patent grafts (1 8 13, 15) while others found progression only in vessels whose grafts have been closed (9) Finally a number of investigators have demonstrated acceleration of lesions in all grafted vessels regardless of graft patency (4, 5 12 14 16) Our findings in the 60 patients reported here are in keeping with the latter investigators showing that progression of the disease in segments proximal to anastomosis occurred in about 50% of all grafted arteries irrespective of graft patency It is, however noteworthy that progression to occlusion was much more frequent in the segments with the most severe obstruction in the preoperative angiogram (75–99% stenosis) than in those that were normal or mildly narrowed at the initial study

The mechanism involved in the occlusion of the proximal segment of grafted coronary arteries is not yet known Some authors (2 7) suggest that the changes represent the natural course of the atherosclerotic process Another possible explanation (5 20) is that the changes are related to a thromboembolic process originating from the suture line Our data neither support nor contradict this assumption Altered pressure–flow relationships due to grafting must also be considered as a cause of progressive narrowing or occlusion of the segment proximal to anastomosis Furuse et al (6) showed in experimental studies that when a bypassed coronary artery is constricted by 50% the flow through that artery falls to less than 5% of the total flow and with more severe stenosis there is a further reduction of flow through the native artery Kakos et al (11) noted in animals a marked decline in flow through the stenotic proximal coronary segment after bypass with open graft Finally the watershed effect reported by Rees (17) may also have contributed to the high rates of occlusion of proximal segments as most grafts were inserted into nonoccluded arteries

There have been suggestions that aortocoronary bypass may accelerate the atherosclerotic process

in segments distal to the site of anastomosis (20) Like other investigators we found that progression distal to the graft anastomosis occurred at a rate almost identical to progression in nongrafted arteries We conclude from this that grafting does not accelerate distal progression This is however correct only as long as the graft is patent Like Griffith et al (9) we found that occlusion of the distal segment was more frequent when the graft was closed The occurrence of graft closure under these circumstances can be related to anastomosing of coronary arteries with poorer run off than assumed at the preoperative study but can also be related to surgical manipulation of the coronary artery itself

## REFERENCES


1 Aldridge H E & Trimble A S Progression of proximal coronary lesions to total occlusion after aortocoronary saphenous vein bypass grafting J Thorac Cardiovasc Surg 62 7 1971

2 Benchimol A Harris C L Fleming H & Desser K B Progression of obstructive coronary artery disease after manipulation of aorto-coronary saphenous vein bypass grafts J Thorac Cardiovasc Surg 68 257 1974

3 Bjork L & Keefie A Estimation of coronary artery stenosis Limitation of present methods Acta Radiol Diag 17 777 1976

4 Bourassa M G Goulet C & Lesperance J Progression of coronary arterial disease after aortocoronary bypass grafts Circulation (Suppl) III 127 1973

5 Frick M H Valle M Harjola P T & Korhola O Changes in native coronary arteries after coronary bypass surgery Am J Cardiol 36 744 1975

6 Furuse A Klopp E H Brawley R K & Gott V L Hemodynamics of aorto to coronary artery bypass Experimental and analytical studies Ann Thorac Surg 14 282 1972

7 Gensini G G Esente P & Kelly A Natural history of coronary disease in patients with and without coronary bypass graft surgery Circulation (Suppl) II 98 1974

8 Glassman E Spencer F C Krauss K R Weisinger B & Ison O W Changes in underlying coronary circulation secondary to bypass grafting Circulation (Suppl) II 80 1974

9 Griffith L S Achuff S C Conti C R Humphries J O Brawley R K Gott V L & Ross R S Changes in intrinsic coronary circulation and segmental ventricular motion after saphenous vein coronary bypass surgery N Engl J Med 288 589 1973

10 Judkins M P Selective coronary arteriography Part I A percutaneous transfemoral technic Radiology 89 815 1976

11 Kakos G S Oldham H N Jr Dixon S H Jr Davis R W Hagen P O & Sabiston D C Jr Coronary artery hemodynamics after aorto-coronary artery vein bypass An experimental study evaluation J Thorac Cardiovasc Surg 63 849 1972

12 Levine J A Bechtel D J Gorlin R Cohn P F Herman M V Cohn L H & Collins J J Jr Coronary artery anatomy before and after direct revascularization surgery Clinical and cinearteriographic studies in 67 patients Am Heart J 89 561 1975

13 Malinow M R Kremkau E L Kloster F E Bonchek L I & Rosch J Occlusion of coronary arteries after vein bypass Circulation 47 1211 1973

14 Maurer B J Oberman A Holt J H Jr Kouchokos N T Hones W B & Russel R O Jr Changes in grafted and non grafted coronary arteries following saphenous vein bypass grafting Circulation 50 293 1974

15 McLaughling P R Berman N D Morton B C McLoughling M D Aldridge H E Adelman A H Goldman B S Trimble A S & Morch J E Saphenous vein bypass grafting Changes in native circulation and collaterals Circulation (Suppl) I 66 1975

16 Pasternak R Cohn K Selzer A & Langston M F Enhanced rate of progression of coronary artery disease following aortocoronary saphenous vein bypass surgery Am J Med 58 166 1975

17 Rees S The watershed a factor in coronary vein graft occlusion Br Heart J 38 197 1976

18 Ross A M Hammond G L Cohen I S & Wolfson S Angiographic evaluation of saphenous vein bypass grafts Artifactual occlusion caused by dual sources of flow Am J Cardiol 391 384 1977

19 Snedecor G W Statistical methods 5th ed Iowa State College Press Ames Iowa 1956

20 Vlodaver Z & Edwards J E Pathological changes in aortic coronary arterial saphenous vein grafts Circulation 44 719 1971

# Failure of Chlorothiazide to Improve Urinary Concentrating Capacity in Lithium-Treated Patients

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ABSTRACT Seven patients on long term lithium treatment were given chlorothiazide for three days The urinary concentrating capacity did not change during the study, but serum potassium decreased significantly Thus chlorothiazide does not seem to produce an effect on a moderately decreased concentrating capacity during long term lithium treatment

*Key words* chlorothiazide urinary concentrating capacity lithium diabetes insipidus

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Kidney lesions have been found following long term lithium treatment (8) Polyuria in connection with lithium treatment has been known for several years (1 6) and most patients on a long term lithium regimen have an impaired urinary concentrating capacity (3) These side effects of lithium are often troublesome to the patient and may constitute a potential hazard (4) Chlorothiazide has a beneficial effect in renal diabetes insipidus (5) and has been reported to reduce the polyuria and improve the urinary concentrating capacity in lithium treated patients (7 12) The effect of chlorothiazide seems to appear within two or three days of treatment (7) Chlorothiazide treatment might thus be of value for patients with impaired urinary concentrating capacity due to lithium The aim of the present study was to test the effect of chlorothiazide on the concentrating capacity in such patients

## METHODS

Seven in patients at Umedalen Hospital were studied Their mean age was 46 years They had been treated with lithium for more than 2 years (mean 4 5) The daily dose of lithium was not changed during the study Chlorothiazide 0 5 g daily was given at 4 p m for three days (I III) The serum concentration of lithium sodium potassium chloride and creatinine were determined at 8 a m on the first four days (I IV) At the same time the urinary concentrating capacity was estimated (days I IV) with the intranasal DDAVP test as previously described (2) DDAVP (Minirin® Ferring Malmo Sweden) was administered intranasally in a dose of 40 μg and the urine osmolality was determined three hours later

For statistical calculations Wilcoxon s test for paired observations was used with a significance level of $p<0\ 05$

## RESULTS

The urinary concentrating capacity remained at the same level from day to day serum potassium decreased significantly ($p<0\ 02$) in all patients serum lithium did not change significantly (Table I) The serum levels of chloride sodium and creatinine were normal at all determinations and did not change

## DISCUSSION

There are several possible explanations for our failure to reproduce the results of earlier studies on the effect of chlorothiazide upon the concentrating capacity in lithium treated patients Our patient series was small and the observation time on chlorothiazide was short Other investigators however have reported an effect of chlorothiazide on the concentrating capacity within three days (7 12) Initially we planned to examine a larger number of patients for a longer time but we interrupted our study in view of the observed decrease in s potassium and because no effect was observed on the concentrating capacity Another explanation might be that our patients had too good concentrating capacities As far as we know no study has been performed to determine whether an effect of chlorothiazide on lithium induced impairment of the con

Table I *Mean urinary concentrating capacity serum potassium and serum lithium*

Chlorothiazide 0 5 g was given on days I III The examination on day I was performed before chlorothiazide

| Day | U-osmolality ($mOsm/kg\ H_2O$) Mean | Range | S-potassium (mmol/l) | S-lithium (mmol/l) |
|---|---|---|---|---|
| I | 642 | 215–819 | 4 1 | 0 7 |
| II | 656 | 358–852 | 3 8 | 0 6 |
| III | 642 | 446–851 | 3 6 | 0 7 |
| IV | 569 | 391–847 | 3 6 | 0 7 |

centrating capacity may be restricted to the lowest concentrating capacities It is also possible that diuretics might have an effect upon the basal concentrating capacity without affecting the maximal concentrating capacity

Diuretics have been found to reduce the renal lithium clearance (11) subsequently increasing the risk of lithium intoxication (9 10) Hypokalemia might contribute to induce renal lesions In our opinion diuretics should be used very restrictively or not at all in combination with lithium Probably such a policy would not cause any serious problems diuretics are generally used in hypertension or congestive heart failure because of their diuretic effect but when combined with lithium they have the opposite effect according to previous investigators (7 12) We were unable to find any effect of chlorothiazide on the concentrating capacity The diuresis was not examined in this study so we cannot exclude the possibility that the diuresis might diminish during chlorothiazide therapy without affecting the concentrating capacity

## REFERENCES


1 Angrist B M Gershon S Levitan S J & Blumberg A G Lithium induced diabetes insipidus-like syndrome *Compr Psychiatry 11 141 1970*
2 Asplund K Wahlin A & Rapp W D D A V P test in assessment of renal function during lithium therapy Lancet 1 491 1979
3 Bucht G & Wahlin A Impairment of renal concentrating capacity by lithium Lancet 1 778 1978
4 — Impairment of renal concentrating capacity by *lithium Lancet 2 560 1978*
5 Crawford J D Kennedy G C & Hill L E Clinical results of treatment of diabetes insipidus with drugs of the chlorothiazide series N Engl J Med 262 737 1960
6 Editorial Lithium-induced diabetes insipidus Br Med J 2 726 1972
7 Forrest J N Jr Cohen A D Torretti J Himmelhoch J M & Epstein F H On the mechanism of lithium induced diabetes insipidus in man and the rat J Clin Invest 53 1115 1974
8 *Hestbech J Hansen H E Amdisen A & Olsen S* Chronic renal lesions following long term treatment with lithium Kidney Int 12 205 1977
9 Lutz E G Lithium toxicity precipitated by diuretics J Med Soc NJ 72 439 1975
10 *Mac Fie A C* Lithium poisoning *precipitated* by diuretics Br Med J 1 516 1975
11 Petersen V Hvidt S Thomsen K & Schou M Effect of prolonged thiazide treatment on renal lithium clearance Br Med J 3 143 1974
12 Robak O H & Saetermo R Behandling av *litiumindusert polyuri Tidsskr Nor Laegeforen 95* 436 1975

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# Immunological and Hematological Abnormalities in Chronic Alcoholism

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ABSTRACT Thirty two chronic alcoholics were studied immunologically and hematologically on the first hospital day after a period of excessive alcohol consumption No patient had any signs of severe liver disease All patients were tested for delayed skin hypersensitivity to PPD and mumps antigens Quantitation of immunoglobulins and routine hematological tests were also performed Fifteen and 23 patients did not respond to PPD and mumps antigens respectively, compared to about 10 and 40% of anergic controls Anergic patients had lower haptoglobin levels than skin positive patients Elevated IgM values were common Thrombocytopenia was the most common (41%) hematological disturbance while 19% of the patients were anemic

*Key words* alcoholism anemia thrombocytopenia delayed skin hypersensitivity

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Patients with chronic alcoholism have an increased susceptibility to infection Pneumonia of all bacterial types is the most common complication (7) Alcoholics are also more prone to develop tuberculosis (10) and other infections (14) The cause of the increased incidence of infections is not fully understood but in recent reviews multiple factors have been suggested (14 15) Impairment of delayed hypersensitivity (16) and leukopenia in combination with other blood dyscrasias (4 5) have been described There seems to be no impairment of antibody production (14)

In the present study delayed hypersensitivity to PPD and mumps antigens immunoglobulins and peripheral blood cell counts were investigated in 32 consecutive abusers of alcohol Differences between anergic and skin positive patients are discussed

## STUDY POPULATION AND METHODS

### *Patients*

Thirty two consecutive patients 8 females and 24 males with chronic alcoholism admitted to the Department of Clinical Alcohol and Drug Research at Karolinska Hospital were studied All had an acute alcohol debauch and were hospitalized for detoxication Their mean age was 44 years (range 25–70) A history of excessive consumption during the last ten years was given by all patients The duration of the period of alcohol abuse preceding admission exceeded 20 days in all cases No patient had any evidence of severe liver insufficiency as judged by normal prothrombin and albumin values and lack of ascites

### *Controls*

Forty age matched healthy persons from the laboratory and hospital staff were used as controls in skin tests for delayed hypersensitivity

### *Methods*

Purified protein derivate of tuberculin (2 TU PPD) (Statens Bakteriologiska Laboratory Stockholm) and mumps antigen (Eli Lilly & Co Indianapolis In ) were used Patients received intradermal injections of 0 1 ml of the antigens on the volar surface of the forearm Skin reactions were evaluated after 48 hours by measuring the crossed diameters of the induration A mean diameter of 6 mm or more was considered positive Immunoglobulins were quantitated by the rocket technique according to Laurell Other analyses were performed according to routine procedures

### *Statistical analysis*

Conventional methods were used for the calculation of the arithmetic mean and S E M To test the hypothesis of two means being equal against a two-sided alternative Student s *t* test was used As a measure of association for each pair of variables Pearsons s product moment correlation (*r*) was chosen

Table I *Clinical variables in relation to PPD skin reactivity*

| | Mean ± S E M | | | |
|---|---|---|---|---|
| | PPD positive (n=17) | PPD-negative (n=15) | Normal range | p value |
| Age (y) | 41 0±3 7 | 44 7±3 3 | | n s |
| S-amylase (µkat/l) | 3 5±0 3 | 3 8±0 4 | 1 1–5 0 | n s |
| S albumin (g/l) | 41 3±1 2 | 40 6±1 5 | 37–52 | n s |
| S-γ GT (µkat/l) | 6 5±2 4 | 2 8±0 7 | <1 0 | n s |
| S ALAT (µkat/l) | 1 2±0 2 | 0 9±0 2 | <0 7 | n s |
| Hb (g/l) | 142 5±3 1 | 150 1±4 1 | ♀ 120–150<br>♂ 140–170 | n s |
| WBC (×10⁹/l) | 9 1±0 7 | 6 7±0 6 | 4 0–9 0 | n s |
| Platelets (×10⁹/l) | 200±28 | 160±21 | 150–400 | n s |
| IgG (g/l) | 10 9±0 8 | 11 2±0 5 | 7 0–15 0 | n s |
| ESR (mm/h) | 10±3 | 6±1 | ♀ 2–15<br>♂ 2–10 | n s |
| S-haptoglobin (g/l) | 2 9±0 2 | 2 3±0 2 | 0 4–2 5 | <0 05 |

n s = Not significant

## RESULTS

### *Skin tests*

Seventeen patients (53%) and 36 controls (90%) reacted positively to 2 TU PPD (2) Only nine patients (28%) had a positive skin test to mumps antigen the corresponding figure in the controls being 60% Anergy to both mumps and PPD was found in 13 patients (41%) PPD-negative patients had a mean age of 44 7 years compared to 41 0 years in PPD reactive patients (Table I) No sex related difference in delayed skin hypersensitivity was obsevered There were no differences in amylase albumin γ glutamyltransferase (γ GT) alanine aminotransferase (S ALAT) Hb WBC platelets or IgG between PPD-positive and PPD-anergic patients (Table I) However serum haptoglobin was increased in seven patients reacting to both antigens (3 2±0 3 g/l) compared to 13 skin negative patients (2 2±0 2 g/l) (mean±S E M $p<0\ 02$) A similar difference was found between PPD-positive and PPD negative patients (Table I) ESR was much the same in the two groups (Table I)

### *Serum immunoglobulins*

Two patients had subnormal IgG concentrations (6 7 and 6 8 g/l respectively) and two had slightly elevated IgG values (15 2 and 18 8 g/l respectively) with a concomitant increase in IgM Out of 11 tested patients 5 had high IgM concentrations (>3 8 g/l) while IgA was within the normal range in all tested patients No common denominator was found among patients with abnormal IgG or IgM values

### *Hematological findings*

Six patient (all men) had Hb values below the normal range They did not show any signs of gastrointestinal or other bleeding Thrombocytopenia was very frequent Thirteen patients (41%) had platelet counts below 150×10⁹/l A positive correlation was found between Hb values and platelet counts ($p<0\ 05$) Neither granulocytopenia nor lymphocytopenia was observed in any patient On the other hand eight patients had leukocyte counts above the normal range without any signs of infectious disease and with a normal ESR There was a strong association between WBC and platelet counts ($r=0\ 68$ $p<0\ 001$) No statistically significant correlation was found between Hb values and WBC counts ($r = 0\ 25$)

### *Other tests*

Twenty three patients (72%) had elevated γ GT and 18 (56%) had elevated S ALAT There was a slight but statistically insignificant correlation between these two variables ($r = 0\ 31$) Platelet counts were inversely correlated with γ GT ($r = 0\ 42$ $p<0\ 05$) No patient showed a severe hypoalbuminemia (<30 g/l) although the mean value of all patients was 41 g/l (normal range 37–52) Eighteen patients (56%) displayed high haptoglobin values (>2 5 g/l)

## DISCUSSION

Impaired delayed skin hypersensitivity to recall antigens and failure to respond to DNCB sensitization has been reported in patients with alcoholic liver disease (1) These patients often show a marked increase in the total gammaglobulin concentration mainly due to an increased production of IgG and IgM (14) Hematological abnormalities—such as anemia thrombocytopenia and granulocytopenia—are also frequent (4 5 17)

In the present study 32 patients with chronic alcoholism but with no or only minor signs of liver insufficiency have been tested Despite preserved liver functions there was a subnormal frequency of responses to PPD and mumps antigens This defect in cell mediated immunity may have various causes *Malnutrition is known to impair cell* mediated immunity both in vivo and in vitro (12) Granulocyte mobilization into skin abrasions is significantly inhibited by acute alcohol intoxication (6) In order to develop a delayed skin hypersensitivity reaction normal macrophages must also be present which could imply some functional macrophage defects Intrinsic lymphocyte abnormalities may also be responsible for the immunodeficiency This can be elucidated by in vitro stimulation of isolated lymphocytes with PPD and a battery of mitogens Furthermore inhibitory serum factors may depress the in vivo reaction to recall *antigens (8) There is usually a high correlation* between the in vitro and in vivo responses to PPD (1 9) However the presence of inhibitory serum factors can be suspected when the in vitro response to PPD is preserved but the in vivo response is extinguished Plasma from cirrhotic patients has been shown to inhibit the lymphocyte response to PHA (11) Chronic alcoholics seem to have normal blood T lymphocyte counts (13) Moreover no correlation has been found between the number of T cells and the size of the PPD skin reaction (13) Degenerative skin changes which have been suggested as a partial explanation for the anergy found in the elderly (2) are less likely to occur in these patients

The association between haptoglobin and skin reactivity is rather difficult to explain but may reflect a reactive state in skin positive patients

Only minor abnormalities of the immunoglobulin pattern were recorded in the patients Elevated IgM levels were found in 45% of the tested patients which is in good accordance with the results of Berenyi et al (1)

In a recent study of chronic alcoholics with a low frequency of hepatic cirrhosis (<10%) Wallerstedt (17) observed thrombocytopenia (<100×10⁹/l) in 10% of his patients Of the present patients 41% had platelet counts below 150×10⁹/l which implies a potential risk of bleeding in this patient category The thrombocytopenia seems to be caused by a direct depression of the bone marrow and a shortened platelet survival time (5 17) After a week s hospitalization a significant increase in platelet counts was observed (17) Anemia and leukopenia are also frequent in chronic alcoholism Since erythrocytes have a long lifespan the influence of alcohol must be of long duration to induce a decrease in the Hb content If hepatosplenomegaly is *present an increased destruction of cells will* furhter aggravate the cytopenias Since there was a good correlation between Hb and platelet counts and these counts and WBC but not between Hb and WBC partly different mechanisms may lie behind the cytopenias

Finally the frequency of anemic patients (19%) in the present study may be falsely low since most patients are hemoconcentrated on admission on account of alcohol induced diuresis and perhaps insufficient fluid intake

## REFERENCES


1 Berenyi M R Straus B & Krus D In vitro and in vivo studies of cellular immunity in alcoholic cirrhosis Digestive Diseases 19 199 1974
2 Bjorkholm M Immunodeficiency in Hodgkin s disease and its relation to prognosis Scand J Haematol (Suppl) 33 1978
3 *Bjorkholm M Holm G Johansson B & Mellstedt* H T lymphocyte deficiency following adult thymectomy in man Scand J Haematol 14 210 1975
4 Bottiger L E Blood damage from alcohol Lakartidningen 73 4099 1976
5 Eichner E R The hematologic disorders of alcoholism Am J Med 54 621 1973
6 Gluckman S J & McGregor R R Effect of acute *alcohol intoxication on granulocyte mobilization and* kinetics Blood 52 551 1978
7 Hoeprich P D Bacterial pneumonias in infectious diseases (ed P D Hoeprich) p 311 Harper & Row Hagerstown 1972
8 Holm G Angelin B Bjorkholm M Ericsson P Mellstedt J & Pettersson D Immunosuppressive serum factors and lymphocyte deficiency in Hodgkin s disease J Clin Lab Immunol 1 269 1979

9 Holm G Mellstedt H Bjorkholm M Johansson B Killander D Sundblad R & Soderberg G Lymphocyte abnormalities in untreated patients with Hodgkin s disease Cancer 37 751 1976
10 Holmdahl S G Four population groups with relatively high tuberculosis incidence in Goteborg 1957–1964 Scand J Respir Dis 18 308 1967
11 Hsu C C S & Leevy C M Inhibition of PHA stimulated lymphocyte transformation by plasma from patients with advanced alcoholic cirrhosis Clin Exp Immunol 8 749 1971
12 Jose D G & Good R A Immune resistance and malnutrition Lancet 1 314 1972
13 Kvetny J T lymphocyte determination in tuberculosis Scand J Respir Dis 58 181 1977
14 Smith F E & Palmer D L Alcoholism infection and altered host defenses A review of clinical and experimental observations J Chron Dis 29 35 1976
15 Straus B & Berenyi M R Infection and immunity in alcoholic cirrhosis Mt Sinai J Med NY 40 631 1973
16 Straus B Berenyi M R Huang J M & Straus E Delayed hypersensitivity in alcoholic cirrhosis Digestive Diseases 16 509 1971
17 Wallerstedt S The usefulness of routine tests in alcoholics Lakartidningen 76 971 1979

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# Serum Ferritin and Bone Marrow Iron in Non-Dialysis, Peritoneal Dialysis and Hemodialysis Patients with Chronic Renal Failure

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**ABSTRACT** Serum ferritin was measured by immunoradiometric assay, and stainable bone marrow iron was assessed semiquantitatively in 38 patients (19 ♂ 19 ♀) with chronic renal failure (7 non dialysed uremic patients, 14 patients on regular peritoneal dialysis and 17 patients on regular hemodialysis) Serum ferritin was also measured in 60 healthy subjects (30 ♂ 30 ♀) There was a good correlation ($p<0.001$) between serum ferritin and marrow iron in the total group of uremic patients Patients without stainable marrow iron ($n=19$) had a geometric mean serum ferritin of 51 ng/ml (range 6–136), mean serum ferritin in patients with "normal" marrow iron ($n=14$) was 326 ng/ml (range 112–1 120) and in patients with slightly increased marrow iron ($n=5$) 634 ng/ml (range 480–960) Male patients had higher mean serum ferritin (244 ng/ml) and higher marrow iron score than female patients (76 mg/ml) ($p<0.01$) All patients had higher mean serum ferritin (141 ng/ml range 6–1 120) than the healthy subjects (46 ng/ml, range 5–285) ($p<0.001$) Patients with serum ferritin levels of <112 ng/ml had no stainable marrow iron, while those with ferritin levels of >136 ng/ml had adequate marrow iron stores The serum ferritin concentration appears to be a reliable indicator of stainable marrow iron content in uremic subjects Regular monitoring of serum ferritin may be a guide to appropriate iron supplementation and reduces the need for repeated marrow punctures in the assessment of iron status in chronic renal failure

*Key words* kidney failure chronic peritoneal dialysis hemodialysis ferritin bone marrow examination iron metabolism

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Iron deficiency is a frequent complication in patients with chronic renal failure This applies both to non dialysed uremics (11) to patients on regular peritoneal dialysis (10) and especially to patients on regular hemodialysis (12) The main factors behind this deficiency are increased iron losses due to blood sampling blood losses connected with the dialysis procedure and increased gastrointestinal and menstrual blood losses Furthermore the protein restricted diet of these patients has a low iron content Accordingly there exists a basic need for iron supplementation as previously emphasized (10 11 12)

It is difficult however to individualize prophylactic iron supplementation because iron losses vary greatly from one patient to another Sequential monitoring of iron status is necessary in these patients in order to adjust iron treatment so that overt deficiency or overloading can be avoided Serum iron plasma transferrin and plasma transferrin saturation give little indication of the iron balance in patients with chronic renal failure and are poorly correlated to stainable bone marrow hemosiderin iron (10 11 12) The stainable marrow iron is considered the most reliable guide to iron status (17) but repeated marrow aspiration is an undesirable procedure However serum ferritin levels have been found to correlate well with mobilizable iron stores and semiquantitative assessment of stainable marrow iron in normal subjects (6 9 16) Furthermore correlations between serum ferritin and stainable marrow iron and iron absorption have been demonstrated in hemodialysis patients (1 2 4 5 13)

The aim of the present study was to evaluate whether the serum ferritin concentration is a clinically useful indicator of stainable marrow iron in various groups of uremic subjects

Table I *Serum ferritin stainable bone marrow iron and hematological data on 38 patients with chronic renal failure*

| | No of pats ♂ | ♀ | Hb (mmol/l) | Serum iron (μmol/l) | Plasma transferrin (μmol/l) | Plasma transferrin saturation (%) | Menstruation (no of ♀) | Oral iron treatment for ≥6 mo (no of pats) |
|---|---|---|---|---|---|---|---|---|
| Group I (*n* = 7) | 2 | 3 | 6 1±1 4 | 10 8± 4 0 | 26 8±4 0 | 21 0±10 2 | | |
| | | 1 | 6 9 | 37 2 | 25 6 | 72 7 | | 1 |
| | 1 | | 5 0 | 7 0 | 30 0 | 11 7 | | |
| *Total* | *3* | *4* | *6 0±1 2* | *14 0±10 8* | *27 1±3 5* | *27 1±22 0* | | |
| Group II (*n* = 14) | | 5 | 5 7±1 0 | 9 8± 2 8 | 27 8±5 5 | 18 1± 6 2 | 4 | 1 |
| | 3 | 5 | 6 1±1 1 | 13 0± 6 2 | 21 4±3 3 | 29 5±10 7 | 1 | 4 |
| | | 1 | 6 0 | 15 2 | – | – | | 1 |
| Total | 3 | 11 | 5 9±1 0 | 12 0± 5 1 | 24 1±5 3 | 24 8±10 5 | | |
| Group III (*n* = 17) | 6 | 3 | 5 4±1 7 | 12 9± 4 1 | 26 4±4 3 | 24 6± 8 0 | 3 | 4 |
| | 4 | 1 | 4 3±1 3 | 20 3±10 0 | 18 9±4 0 | 51 0±15 6 | 1 | 4 |
| | 3 | | 4 4±0 3 | 23 8± 7 0 | 18 2±1 6 | 65 8±21 0 | | 3 |
| Total | 13 | 4 | 4 9±1 2 | 18 0± 8 2 | 23 1±5 3 | 42 2±22 9 | | |

## PATIENTS AND METHODS

The study comprised 38 patients divided into three groups

*Group I* Seven patients (3♂ 4♀) mean age (±S D ) 46±10 years with non-dialysed chronic renal failure and 24-hour endogenous creatinine clearance of 6 9±2 5 ml/min

*Group II* Fourteen patients (3♂ 11♀) mean age 46±10 years on regular home peritoneal dialysis during 2–23 months with a creatinine clearance of 2 1±1 4 ml/min Dialysis was performed for 5 hours 6 days weekly using a manual system (3)

*Group III* Seventeen patients (11♂ 6♀) mean age 43±11 years on regular hemodialysis during 3–51 months for 6 hours twice weekly using the Gambro-Lundia® dialysis filter and with a creatinine clearance of <0 5 ml/min

None of the patients had hepatic dysfunction or overt infection All were on a protein restricted diet and received multivitamin supplementation except vitamin $B_{12}$ and folate None of the patients had received parenteral iron but some in each group were treated with oral iron as ferrous fumarate 400 mg (132 mg elemental $Fe^{2+}$) thrice daily and 7 patients in group III had received blood transfusions (Table I)

*Controls* Blood samples for estimation of serum ferritin were drawn from 60 apparently healthy subjects (30♂ 30♀) aged 20–50 years

Hb serum iron plasma transferrin and percentage plasma transferrin saturation were assessed by previously described procedures (8) Serum ferritin was measured by a two-site immunoradiometric assay using antibody coupled paper discs as a solid phase The method has been described in detail elsewhere (14)

Bone marrow specimens were obtained by sternal puncture and stained for iron with Prussian blue whereafter the stainable hemosiderin iron content was assessed *blindly* and graded semiquantitatively in 4 classes by one of the authors as follows 0 = no stainable iron 1+ = normal 2+ = slightly increased 3+ = greatly increased

The distribution of serum ferritin values was skewed as previously reported (6 9 15) and logarithmic transformation resulted in a normal distribution Consequently the significance of (ferritin) differences between two groups

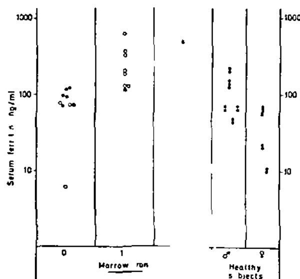



*Fig 1* Relation between serum fe[illegible] d stainable bone marrow iron in non-dialysis (■) [illegible] al dialysis (O) and hemodialysis (●) patients wit[illegible] c renal failure and serum ferritin values in healthy [illegible]

| lo ansfu ons | | Serum fermtin (ng/ml) | | Bone marrow ron (grade) |
|---|---|---|---|---|
| o of ats | Units | Geometric mean | Range | |
| | | 4 | 30–72 | 0 |
| | | 11? | | 1+ |
| | | 5 5 | | ?+ |
| | | 70 | 30–525 | |
| | | 29 | 6–76 | 0 |
| | | 224 | 1 8–610 | 1+ |
| | | 530 | | ?+ |
| | | 114 | 6–610 | |
| | 5 and 33 | 79 | 20–136 | 0 |
| | 16 19 and 35 | 470 | 480–1 1 0 | 1+ |
| | 31 and 34 | 717 | 480–960 | ?+ |
| | | 225 | 0–1 1 0 | |

was evaluated by Student s *t* test performed on log arithms and geometric means are quoted Kruskal Wall s test was employed to determ ne the s gn f cance of d fferences between three nterdependent groups and Spearman s rank correlat on coeff c ent (*r* ) to assess s g nif cant correlat ons

## RESULTS

The results are summarized n Tables I and II and Figs 1 and 2

All three groups of patients demonstrated an ex cellent correlat on between sta nable marrow ron and serum fernt n (Kruskal Wall s test *H* 27 0 $p<0\ 001$) Pat ents hav ng no sta nable marrow iron (*n* 19) had a geometric mean serum fernt n of 51 ng/ml (Table II) Pat ents w th grade 1+ marrow ron (*n* 14) had a mean serum ferritin of 326 ng/ml and those w th grade 2+ marrow ron (*n* 5) had a mean serum fernt n of 634 ng/ml All pat ents with serum ferritin levels of <112 ng/ml had no stainable marrow iron wh le all those w th levels of >136 ng/ml had adequate marrow iron stores

Geometric mean serum ferritin in non d alysed urem cs was 70 ng/ml n patients on peritoneal dialys s 114 ng/ml and in hemod alysed patients ?25 ng/ml In the three groups of patients comb ned geometric mean serum fernt n was 141 ng/ml which is s gn f cantly h gher than in the group of 60 healthy subjects (46 ng/ml) ($p<0\ 001$)

Serum fernt n was s gn ficantly h gher in males than in females among both patients and healthy subjects ($p<0\ 01$ and $p<0\ 001$) Marrow iron status was also better n male than in female pat ents Eight males and 11 females had grade 0 7 males and 7 females grade 1+ and 4 males and 1 female grade 2+ marrow iron

There were s gn f cant correlat ons bet veen serum ferritin and serum iron ($r_s$ 0 49 $p<0\ 001$) plasma transferrin ($r_s$ 0 60 $p<0\ 001$) and plas ma transferrin saturat on ($r_s$ 0 64 $p<0\ 001$) No correlat on existed between serum iron and marrow iron (Kruskal Wall s *H* 4 5 *p* 0 105) How ever both *plasma transferrin and transferrin satura* t on were s gn ficantly correlated to sta nable mar row ron (*H* 11 8 *p* 0 002 and *H* 12 0 *p* 0 00?) but as shown n Fig 2 there was cons der able overlapp ng between the groups

## DISCUSSION

The serum ferritin concentrat on n healthy subjects is directly related to available body ron stores as measured by quantitat ve phlebotomy (16) Com parisons between serum ferritin and semiquantita t ve assessment of sta nable marrow ron have also

Table II *Ser ferr t n n 38 pat ents tl cl ro c re ıal fa l re and ı 60 health) s bjects*

| | Pat ents | | | | | | Heal hy subjects | | |
|---|---|---|---|---|---|---|---|---|---|
| | Marrow ron grade) | | | | | | | | |
| | 0 | 1+ | 2+ | ♀ | ♂ | Total | ♀ | ♂ | Total |
| Serum fernt n (ng/ml) | | | | | | | | | |
| Geometric mean | 51 | 3 6 | 634 | 76 | 44 | 141 | ?5 | 8? | 46 |
| Arithmet c mean | 65 | 4 9 | 659 | 154 | 401 | 77 | 35 | 99 | 67 |
| Range | 6–136 | 11? 1 1 0 | 480–960 | 6–610 | 30–1 1 0 | 6–1 1 0 | 5 160 | 19– 85 | 5– 85 |
| No of pats | 19 | 14 | 5 | 19 | 19 | 38 | 30 | 30 | 60 |

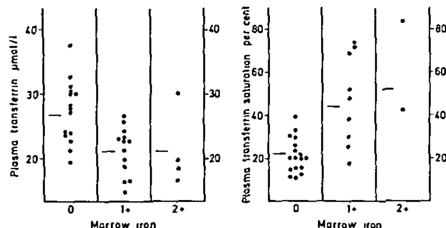



*Fig 2* Relation between plasma transferrin and transferrin saturation and stainable bone marrow iron in the 38 patients with chronic renal failure

demonstrated a relation between these two indices of iron stores (9) In the present study a good correlation was found between serum ferritin and stainable marrow iron in non-dialysed uremics and in patients on regular peritoneal dialysis or regular hemodialysis It appears that serum ferritin determinations give a reliable estimation of marrow iron stores in patients with chronic renal failure

Iron deficiency is a potential risk in uremic patients (10 11 12) As the amount of iron needed varies greatly from one patient to another supplementation must be guided by parameters which are representative for body iron stores Repeated marrow aspirations are clearly undesirable in these patients Furthermore serum iron is uncorrelated to stainable marrow iron and the correlations between plasma transferrin and transferrin saturation and marrow iron stores are vague and have too great an overlap to be of clinical value in the individual patient However the introduction of serum ferritin measurements has now made it possible to estimate marrow iron depots with an accuracy which is adequate for clinical purposes and thereby to individualize iron therapy

Our results are in good accordance with previous reports by other authors demonstrating a significant correlation between serum ferritin and marrow iron both in hemodialysis patients (1 2 5 13) and in non-dialysed uremics (1)

Patients without stainable marrow iron had an arithmetic serum ferritin level of 65 ng/ml (range 6–136) while those with normal iron stores had a mean level of 429 ng/ml (range 112–1 120) (Table II) These figures are in agreement with the results of Hussein et al (5) who found an arithmetic mean serum ferritin level of 42 ng/ml (range 10–127) in 4 hemodialysed patients without marrow iron and a mean level of 387 ng/ml (range 120–740) in 6 patients with normal iron stores Furthermore Mirahmadi et al (13) measured an arithmetic mean serum ferritin level of 35 ng/ml (range 5–100) in 9 hemodialysed patients with absent or reduced marrow iron

Serum ferritin concentrations in the healthy subjects were within the normal range reported by others (5 6 13 15 16) and the levels were distinctly higher in males than in females In the uremic patients ferritin levels were likewise higher in males than in females in accordance with the better iron status in the former group

The uremic patients had significantly higher serum ferritin levels than the healthy subjects Whether this can be explained by differences in marrow iron stores rather than by other factors (7) remains unclarified as marrow iron was not assessed in the latter group

The presented results furthermore emphasize the indication for iron supplementation to uremic patients Altogether 19 of the 38 patients had absent or reduced marrow iron stores Twenty patients received no iron treatment and 14 (70%) of these had no stainable marrow iron whereas only 5 (28%) of the 18 iron treated patients had negative marrow iron staining

On the basis of our findings it appears that patients with serum ferritin levels of <110 ng/ml probably have reduced marrow iron stores whereas patients with ferritin levels of >135 ng/ml have adequate marrow iron stores The serum ferritin concentration seems adequately to reflect the level of

marrow iron stores and can be a guide to appropriate iron supplementation in patients with chronic renal failure

## REFERENCES


Aljama P Ward M K Pierides A M et al Serum ferritin concentration a reliable guide to iron overload in uremic and hemodialyzed patients Clin Nephrol 10 101 1978

Bealfo R Dallman P R Schoenfeld P Y & Humphreys M H Serum ferritin and iron deficiency in patients on chronic hemodialysis Trans Am Soc Artif Intern Organs 22 73 1976

Dawids S G & Christensen E Chronic home peritoneal dialysis with a simple dialysis system Proc Eur Dial Transplant Assoc 12 149 1976

Eschbach J W Cook J D Scribner B H & Finch C A Iron balance in hemodialysis patients Ann Intern Med 87 710 1977

Hussein S Prieto J O Shea M et al Serum ferritin assay and iron status in chronic renal failure and haemodialysis Br Med J 1 546 1975

Jacobs A & Worwood M Ferritin in serum N Engl J Med 292 951 1975

Konijn A M & Hershko C Ferritin synthesis in inflammation I Pathogenesis of impaired iron release Br J Haematol 37 7 1977

Larsen L & Milman N Normal iron absorption determined by means of whole body counting and red cell incorporation of $^{59}$Fe Acta Med Scand 198 271 1975

9 Lipschitz D A Cook J D & Finch C A A clinical evaluation of serum ferritin as an index of iron stores N Engl J Med 290 1213 1974

10 Milman N Christensen T Bartels U & Larsen L Iron absorption and iron status in patients with chronic uremia on regular peritoneal dialysis Acta Med Scand 205 629 1979

11 Milman N & Larsen L Iron absorption in patients with chronic renal failure not requiring dialytic therapy Acta Med Scand 198 511 1975

12 — Iron absorption in patients with chronic uremia undergoing regular hemodialysis Acta Med Scand 199 113 1976

13 Mirahmadi K S Paul W L Winer R L et al Serum ferritin level Determinant of iron requirement in hemodialysis patients JAMA 238 601 1977

14 Pedersen N S Axelsen N H Bock E & Nørgård Pedersen B A paper disc two-site immunoradiometric assay for ferritin Prot Biol Fluids 24 659 1976

15 Siimes M A Addiego J E & Dallman P R Ferritin in serum Diagnosis of iron deficiency and iron overload in infants and children Blood 43 581 1974

16 Walters G O Miller F M & Worwood M Serum ferritin concentration and iron stores in normal subjects J Clin Pathol 26 770 1973

17 Weinfeld A Iron stores In Iron deficiency (ed L Hallberg H G Harwerth & A Vanotti) pp 329–363 Academic Press London and New York 1970

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# Pregnancy in Patients with Renal Disease

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ABSTRACT The outcome of pregnancy for both mother and child was studied in 30 patients with renal disease Glomerulonephritis had been verified by biopsy before pregnancy in 20 patients, three had severe chronic pyelonephritis two polycystic renal disease and five had undergone a renal transplantation Morphological or clinical preconception predictors of a riskful pregnancy were hypertension and renal functional impairment and in the patients with glomerulonephritis a biopsy finding known to indicate a poor or guarded prognosis in the non pregnant state or as judged from the patient s poor response to corticosteroid therapy However, none of the patients with glomerulonephritis, pyelonephritis or polycystic renal disease who had conceived while their renal function was normal showed any evidence during pregnancy, of a deterioration of this function Among 29 pregnancies in the 20 patients with a history of glomerulonephritis 5 foetuses were lost No abortions occurred in the other patient groups Six children were born to the five patients with a renal transplant All these patients received immunosuppressive therapy during pregnancy No newborn was malformed In one patient with hypertension and renal insufficiency at the start of pregnancy, a progressive renal insufficiency developed and haemodialysis was started after delivery In most instances the counseling of women with pre-existing renal disease who want to have children can be guardedly optimistic

*Key words* glomerulonephritis renal transplantation pregnancy

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In recent years the counsel given to women with a history of renal disease who want to have children has changed considerably (3) Whereas earlier the outlook for such women was considered poor (7 16) recent studies suggest that in the absence of renal functional impairment and hypertension pregnancy does not usually exacerbate or cause a recurrence of the renal disease (11 14 15) An increased incidence of complications during pregnancy and labour has however been reported in women with a history of nephritis (12) The outcome of pregnancy has been shown to correlate moreover not only with renal function and hypertension but also with the type and severity of the underlying renal lesion (14)

The present investigation was undertaken to study the outcome of pregnancy the course of renal disease in the mother as well as the condition of the infant at birth in 29 pregnancies in 20 women with a history of glomerulonephritis In these patients the morphological classification of the renal disorder was determined by microscopic examination of a renal biopsy Women with chronic pyelonephritis

Table I *Outcome of pregnancies in patients with renal disease*

| | No of pats | No of pregnancies | No of live births | No of abortions |
|---|---|---|---|---|
| Chronic glomerulonephritis | 20 | 29 | 25 | 4 (spontaneous)<br>1 (therapeutic) |
| Chronic pyelonephritis | 3 | 3 | 3 | – |
| Polycystic renal disease | 2 | 3 | 3 | – |
| Renal transplantation | 5 | 5 | 6 | – |
| Total | 30 | 40 | 37 | 5 |

Including one pair of twins

Table II *Glomerulonephritis*

N = normal ND = not determined CS = cesarean section TA = therapeutic abortion SA = spontaneous abortion

| Pat no | Year of birth | No of pregnancies | Year of: Onset of renal disease | Year of: Renal biopsy | Before pregnancy: BP | Before pregnancy: U protein (g/l) | Before pregnancy: Response to steroid therapy | During pregnancy: S protein (g/l) | During pregnancy: BP | During pregnancy: U protein (g/l) |
|---|---|---|---|---|---|---|---|---|---|---|
| *Minimal change glomerulonephritis* | | | | | | | | | | |
| 1 | 1956 | I | 1972 | 1974 | N | 5.4–19 3 | Good | 34 | N | 0 3–4 1 |
| 2 | 1950 | I | 1970 | 1970 71 72 | N | 4 3–0 | Good | 74 | N | 0 |
| | | II | | | N | 0 | Good | 63 | N | 0 |
| 3 | 1952 | I | 1973 | 1973 74 | N | 14 6–5 5 | Poor | 59 | 160/110 | 4 4–11 3 |
| *Focal sclerosis* | | | | | | | | | | |
| 4 | 1951 | I | 1967 | 1971 73 75 | N | 0 8–1 1 | Poor | 50 | N | 0 7–4 0 |
| | | II | | | N | 3 0–12 9 | Poor | 42 | N | 4 5–6 8 |
| *Focal glomerulonephritis (IgA nephropathy)* | | | | | | | | | | |
| *5* | *1947* | *I* | *1976* | *1976* | *N* | *0 5–2 0* | – | *ND* | *N* | *0 [illegible]* |
| *Postinfectious glomerulonephritis* | | | | | | | | | | |
| 6 | 1942 | I | 1957 | 1957 71 | N | 0 | – | ND | 170/105 | 0 |
| | | II | | | N | 0 | – | ND | N | 0 |
| 7 | 1946 | I | 1965 | 1966 | N | 0 | – | 63 | N | 0 |
| *Membranous glomerulonephritis* | | | | | | | | | | |
| 8 | 1950 | I | 1970 | 1971 74 | N | 3 0–0 8 | – | ND | N | 0 4–2 5 |
| | | II | | | N | 2 5–1 6 | – | 57 | N | 2 5–1 2 |
| | | III | | | N | 1 6–1 2 | – | 62 | N | 0–1 0 |
| 9 | 1945 | I | 1972 | 1973 74 | N | 0 | Good | 60 | N | 0–2 4 |
| 10 | 1952 | I | 1965 | 1967 68 74 | N | 1 2–3 9 | Good | 40 | N | 2 5–3 2 |
| 11 | 1954 | I | 1973 | 1973 | N | 0 9–2 0 | Poor | 34 | N | 3 2–16 1 |
| 12 | 1946 | I | 1967 | 1967 75 | N | 0 8–1 2 | Poor | ND | N | 0 4–0 6 |
| | | II | | | N | 0 | Poor | 63 | 160/120 | 0 |
| 13 | 1952 | I | 1968 | 1969 78 | N | 2 6 | – | 44 | N | 1 0–4 7 |
| *Proliferative glomerulonephritis* | | | | | | | | | | |
| 14 | 1943 | I | 1964 | 1967 68 74 | N | 0 2–2 6 | – | ND | N | 0 4–0 8 |
| | | II | | | N | 0 7–2 1 | – | ND | N | 0 5–0 9 |
| | | III | | | N | 0 6–1 8 | – | ND | N | 0 4–0 8 |
| 15 | 1948 | I | 1973 | 1973 | N | 0 | – | ND | 150/100 | 0 |
| 16 | 1952 | I | 1963 | 1968 72 74 76 | N | 5 0–7 0 | Poor | 35 | N | 5 0–5.[illegible] |
| *SLE* | | | | | | | | | | |
| 17 | 1952 | I | 1974 | 1974 | 145/100 | 0–1 1 | Good | 65 | 160/110 | 0 |
| 18 | 1957 | I | 1971 | 1971 | N | 0 | Good | ND | N | 0 |
| 19 | 1947 | I | 1975 | 1975 | N | 0 | – | 61 | N | 0 |
| | | II | | | N | 0 | – | 61 | N | 0 |
| *Mixed connective tissue disease* | | | | | | | | | | |
| 20 | 1951 | I | 1972 | 1972 75 | N | 0 | – | 62 | N | 0 |

and polycystic renal degeneration—patients for whom counseling about a planned pregnancy was particularly difficult—and five women with a renal transplant were also included in the study

## PATIENTS AND METHODS

*Glomerulonephritis group*

Our series included 20 women with a history of glomerulonephritis in whom percutaneous renal biopsies had been studied from 7 months to 18 years before pregnancy Only patients from whom a biopsy had been obtained before conception are included in this group of patients

Minimal change glomerulonephritis was characterized by the nephrotic syndrome unaccompanied by any visible morphological changes in renal tissue on light or immunofluorescence microscopy IgA nephropathy was characterized by recurrent haematuria by focal glomerulonephritis

| Mother's age/ year and type of delivery or abortion | Birth weight (g) | Apgar score |
|---|---|---|
| 19/1975 TA | – | |
| 24/1974 CS | 2 550 | 9 |
| 27/1977 CS | 3 100 | 8 |
| 23/1975 SA | – | |
| 23/1974 SA | – | |
| 25/1976 SA | – | |
| 30/1977 CS | 3 100 | 9 |
| 30/1972 N | 3 280 | 9 |
| 33/1975 N | 4 200 | 9 |
| 31/1977 N | 3 250 | 8 |
| 22/1972 N | 3 800 | 9 |
| 24/1974 N | 3 600 | 9 |
| 25/1975 CS | 3 500 | 9 |
| 30/1975 N | 3 600 | 8 |
| 23/1975 CS | 2 170 | 9 |
| 23/1977 CS | 2 000 | 8 |
| 29/1975 SA | – | |
| 31/1977 N | 2 730 | 9 |
| 26/1978 CS | 3 4_0 | 7 |
| 26/1969 N | 3 740 | 9 |
| 27/1970 N | 3 490 | 9 |
| 29/1972 N | 3 560 | 9 |
| 30/1978 N | 4 160 | 6 |
| 24/1976 N | 2 380 | 8 |
| 25/1977 N | 3 230 | 9 |
| 17/1974 N | 2 550 | 8 |
| 30/1977 CS | 3 330 | 5 |
| 32/1979 CS Twins | 2 680 | 9 |
| | 2 780 | 8 |
| 25/1976 CS | 2 470 | 8 |

on light microscopy of the renal biopsy and by mesangial deposits of IgA on immunofluorescence microscopy In the six patients with membranous glomerulonephritis the glomerular basement membrane showed irregular subepithelial projections and moth-eaten thickenings in silver stained sections No staging of the membranous glomerulonephritis was done In five of these patients the diagnosis was aided by a typical immunofluorescence finding In proliferative glomerulonephritis the lesion showed a lobular proliferative pattern and a split basement membrane in silver stained sections Among the three patients with systemic lupus erythematosus (SLE) the renal morphology was consistent with focal glomerulonephritis in one with mesangioproliferative glomerulonephritis in another and with membranous glomerulonephritis in the third

*Chronic pyelonephritis group*

Three patients had chronic pyelonephritis of 8–30 years duration All three had marked renal parenchymal reduction and/or morphological changes in renal papilli consistent with chronic pyelonephritis One patient had a history of recurrent urinary tract infections Her right kidney was hypoplastic and showed only slight excretory function on i v renography and her left kidney showed local papillary destruction The second patient who had had chronic pyelonephritis since the age of 2 showed bilateral renal parenchymal reduction and unilateral local destruction of papilli but symmetrical function on renography The third patient had unilateral parenchymal and papillary deformities caused by recurrent urinary tract infections

*Polycystic renal degeneration group*

Two patients had manifest polycystic disease The kidney sizes at urography were 6×16 cm and 6×14 cm 6 years before pregnancy in the first patient 8×16 5 cm and 7 5×18 5 cm 2 years before the first and 4 years before the second pregnancy in the second patient

*Renal transplantation group*

The five transplanted patients had all been on haemodialysis or peritoneal dialysis before transplantation Three of them had received an A match allograft from living donors one an A match cadaver graft and one a C match cadaver graft All were on continuous treatment with methylprednisolone 4–20 mg/day and azathioprine 100–150 mg/day

The diagnosis of renal disease was based—in addition to the identification of renal morphological features—on a detailed history and on clinical laboratory and roentgenological findings Throughout pregnancy the patients were regarded as "high risk pregnancies and were followed in particular for the presence of oedema for raised blood pressure (BP) serum creatinine and serum total protein levels and for the degree of proteinuria

Our normal range for serum creatinine in the non pregnant state is 53–115 μmol/l Extrapolation from the plasma creatinine values during pregnancy reported by Kuhlback and Widholm (4) gives the following trimester reference values 54–94 34–96 and 21–85 μmol/l for the first second and third trimester respectively

## RESULTS

Table I shows the number of pregnancies and live births among the patients in the four groups Tables II III and IV give clinical and laboratory data on the 30 women grouped according to renal disease or disturbance

had an uncomplicated pregnancy and delivery During pregnancy two patients received prophylactic chemotherapy none had bacteriuria

*Polycystic renal degeneration* (Table III)

During pregnancy patient 24 had no proteinuria or bacteriuria and no impairment of renal function Two months before delivery however hypertension (160/120) developed and she was treated with hydralazine and propranolol Due to imminent foetal asphyxia a cesarean section was performed In patient 25 pregnancies and deliveries were uncomplicated

*Renal transplantation* (Table IV)

Table IV shows the clinical data on the five patients with a renal transplant The interval between renal transplantation and pregnancy ranged from 1 to 5 years Before pregnancy patient 28 had had acute steroids psychosis osteoporosis and bone necrosis and patient 30 had had diabetic retinopathy and diabetic gangrene In patient 27 thrombocytopenia developed during pregnancy

One of the transplanted patients had an uncomplicated spontaneous delivery the other four were delivered by cesarean section In each instance a healthy child was born In one patient transplant function deteriorated markedly Before pregnancy her serum creatinine was 200 μmol/l and creatinine clearance 30 ml/min her BP was kept at 135/100 with moderate antihypertensive therapy During early pregnancy her BP rose to 205/130 and at 24 weeks of gestation it was 260/160 The serum creatinine value gradually rose to 600 μmol/l at delivery A cesarean section was performed due to pre-eclampsia Her twins weighed at birth 1 450 and 990 g Four weeks after delivery the mother reentered haemodialysis treatment A respiratory distress syndrome developed in both infants and their condition was critical for 2–3 weeks but both left hospital in satisfactory health

## DISCUSSION

The purpose of this study was to review our experience with the outcome of pregnancy in patients with a history of renal disease so that our counseling of patients who want to have children might be better informed Outside the scope of this study were patients in whom renal complications including those associated with pre-eclampsia have occurred during pregnancy as well as patients in whom the nephrotic syndrome was first diagnosed during pregnancy or in whom renal histopathology was first identified in a renal biopsy obtained during pregnancy (5)

From their study of the outcome of pregnancy in a large group of women with a history of nephritis Rauramo et al (12) concluded that a crucial factor in the prognosis was the interval between the onset of renal disease and conception If conception occurred during the first 3 years after the onset of renal disease the risk of complications increased For most of our patients this interval had been at least 3 years—a factor that may have contributed to the generally good outlook for our patients both as regards renal disease and outcome of pregnancy We agree with the conclusions of the above authors on the importance of a sufficiently long interval between the onset of an acute immunologically mediated renal disease and conception In two of our patients with glomerulonephritis who had conceived 7 months and 2 2 years respectively after the onset of renal disease (minimal change glomerulonephritis with nephrotic syndrome) the pregnancy was terminated—in one by a therapeutic and in the other by spontaneous abortion The poor prognosis for the second patient was undoubtedly coupled to the obvious severity of the disease as indicated by the patient s poor response to steroid therapy That pregnancy should be approached with great caution by patients who have a renal disease known to have a poor prognosis in the non pregnant state was clearly shown in our series The one patient with focal sclerosis had two spontaneous abortions and the nephrotic syndrome developed during pregnancy in one patient with a proliferative glomerulonephritis that had responded poorly to steroids Similarly a good primary response to steroids in patients with membranous glomerulonephritis suggests a less severe disease process and probably better prognosis for the outcome of a future pregnancy Moreover if the nephrotic syndrome has responded poorly to steroid therapy before pregnancy then a relapse during pregnancy seems likely

Irrespective of their renal disease hypertension developed during pregnancy in a number of patients even in some who had been normotensive before pregnancy Nor was the degree of proteinuria before pregnancy a predictor of hypertension In all our patients with a history of

glomerulonephritis hypertension was controlled by mild antihypertensive agents

Our laboratory data confirm that in women who have even slight proteinuria when they conceive proteinuria increases during pregnancy This increase has been attributed to the acceleration in the glomerular filtration rate rather than to an exacerbation of the renal disease (1 3) In our patients oedema was mild and easily controlled by diuretics

During pregnancy the serum creatinine level can be interpreted as a sign of azotaemia only in the light of the physiology of pregnancy During a normal pregnancy the glomerular filtration rate accelerates markedly and the serum creatinine level drops by 30-50% (4) Thus serum creatinine values accepted as normal for the non pregnant state may be grossly abnormal during the course of pregnancy

Polycystic kidney disease in its adult form is usually diagnosed between the ages of 25 and 40 Thus many women become pregnant before the disease has become manifest According to Landesman and Scherr (6) there is no evidence that pregnancy per se influences the clinical onset or progression of the polycystic disease process in patients free from hypertension and azotaemia In patients who have these clinical signs however the prognosis is considerably worse (8 10) As stated by Oken (11) it is desirable to avoid pregnancies later in the childbearing period when the increased clinical severity of the disease poses a greater threat to the welfare of mother and child

By 1977 the European Dialysis and Transplantation Association had received reports on 79 live births by transplanted women (17) Less than half of these babies had been delivered by cesarean section their weight at birth was lower than average Four of these 79 infants were born with a congenital abnormality The possible teratogenic effect of the immunosuppressive treatment given to transplanted women has raised considerable concern In another review 71% of the pregnancies (therapeutic abortions excluded) of women with renal transplants resulted in fullterm infants (13) In this large series there was no predominant or frequent developmental abnormality compared with the general newborn population In the present series no baby of a transplanted woman showed any physical abnormality However transient chromosome changes of newborns have been observed (13) These chromosome abnormalities disappear with cessation of the azathioprine exposure One woman who had hypertension and impaired renal function when she became pregnant showed a gradual decline in renal function and had to enter the haemodialysis program after delivery In this instance the premature birth of twins precipitated a prolonged respiratory distress syndrome in both infants It thus appears that the same prepregnant parameters i e hypertension and azotaemia are crucial predictors of the outcome of pregnancy also in transplanted women Even deterioration of renal function that begins during pregnancy has proceeded to postpartum death of a mother with a renal transplant (2 9) Two of our patients had had various extrarenal complications some severe during the prepregnant period (Table IV) These observations suggest that even severe complications associated with renal transplantation do not preclude the satisfacotry outcome of pregnancy in transplanted women

In conclusion we believe that in women with a history of renal disease the presence of renal insufficiency and/or hypertension at the start of pregnancy is a clear risk factor for both mother and child Furthermore in women with glomerulonephritis that has a known poor prognosis or has responded poorly to corticosteroid treatment e g some forms of minimal change and proliferative glomerulonephritis a pregnancy is likely to entail complications In most instances the counseling of women with pre-existing renal disease who want to have children can be guardedly optimistic A satisfactory outcome however requires good team work between nephrologist and obstetrician

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## REFERENCES

1 Bucht H Studies on renal function in man with special reference to glomerular filtration and renal plasma flow in pregnancy Scand J Clin Lab Invest (Suppl) 3 1951
2 Caplan R M Dossetor J M & Maughan G B Pregnancy following cadaver kidney homotransplantation Am J Obstet Gynecol 106 644 1970
3 Editorial Pregnancy and renal disease Lancet 2 801 1975
4 Kuhlback B & Widholm O Plasma creatinine in normal pregnancy Scand J Clin Lab Invest 18 654 1966

5 Kuhlback B Widholm O Skrifvars B Nieminen U Pasternack A Tallgren L G & von Knorring J Acute renal failure in pregnancy Acta Obstet Gynecol Scand 46 475 1967
6 Landesman R & Scherr L Congenital polycystic kidney disease in pregnancy Obstet Gynecol 8 673 1956
7 Mackay E V Pregnancy and renal disease A ten year survey Aust NZ J Obestet Gynecol 3 21 1963
8 Millar W G Pregnancy and polycystic disease of the kidneys J Obstet Gynaec Br Emp 60 868 1953
9 Moore T C & Hume D M The period and nature of hazard in clinical renal transplantation II The hazard to transplant kidney function Ann Surg 170 12 1969
10 Morris N Pregnancy complicated by congenital polycystic disease of the kidneys J Obstet Gynaec Br Emp 59 822 1952
11 Oken D E Chronic renal disease and pregnancy A review Am J Obstet Gynecol 94 1023 1966
12 Rauramo L Kasanen A Elfving K & Salmi H Fertility pregnancy and labour in women with a history of nephritis or pyelonephritis Acta Obstet Gynecol Scand 41 357 1962
13 Rudolph J E Schweizer R T & Bartus S A Pregnancy in renal transplant patients Transplantation 27 26 1979
14 Strauch B S & Hayslett J P Kidney disease and pregnancy Br Med J 4 578 1974
15 Studd J W W & Blainey J D Pregnancy and the nephrotic syndrome Br Med J 1 276 1969
16 Tenney B & Dandrow R V Clinical study of hypertensive disease in pregnancy Am J Obstet Gynecol 81 8 1961
17 Wing A J Brunner F P Brynger H Chantler C Donckerwolke R A Gurland H J Hathway R A Jacobs C & Selwood N H Combined report on regular dialysis and transplantation in Europe VIII 1977 Proc Eur Dial Transplant Assoc 15 3 1978

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# Clinical Trial of Prednimustine, Leo 1031 (NSC-134087), in Patients with Non Hodgkin Lymphomata and Chronic Lymphocytic Leukaemia Previously Treated with Steroids and Alkylating Agents

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**ABSTRACT Prednimustine a chlorambucil ester of prednisolone was administered to 16 patients with non Hodgkin lymphomata (NHL) and 14 patients with chronic lymphocytic leukaemia (CLL) all previously treated with steroids and alkylating agents Response was obtained in 8 patients with NHL and 11 patients with CLL Two NHL patients had long lasting complete remissions Median duration of response for lymphomata was 12 weeks for CLL more than 15 weeks Delayed reversible and rather pronounced myelosuppression was the major side-effect observed in median 6 weeks from the start of Prednimustine with a median duration of 4 weeks**

*Key words* Prednimustine non Hodgkin lymphomata CLL

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Prednimustine an ester of chlorambucil with prednisolone in position 21 was synthesized with the aim of obtaining a facilitated binding to and transport across the cellular membrane especially of tumour cells (10) The drug is absorbed incompletely from the gastrointestinal tract following peroral administration (9 11) activated by hydrolysis and excreted mainly by the urinary route (9) In vitro studies have demonstrated that hydrolysis of Prednimustine to chlorambucil and prednisolone takes place in the presence of blood plasma or cell extracts particularly in experiments with immature blast cells from patients with acute leukaemia (18)

Animal experiments have demonstrated activity against a variety of experimental tumours (7) with altered therapeutic index and toxicity as compared with equivalent doses of chlorambucil and prednisolone

In preliminary investigations in humans the drug has so far revealed an antineoplastic effect in a high percentage of patients with chronic lymphocytic leukaemia (CLL) (3 5 8) non Hodgkin lymphomata (NHL) mainly of the lymphocytic type (5 6 8 13 14) and breast cancer (11 15) Activity has also been demonstrated in a smaller number of patients with acute myelocytic leukaemia (1 2) and ovarian carcinoma (12)

The present investigation was carried out as a phase II trial to further elucidate the activity (and possible side effects) of Prednimustine as a single drug in patients with NHL and CLL previously treated with steroids and alkylating agents

## PATIENTS AND METHODS

Sixteen patients with biopsy proven NHL in clinical stages III and IV and 14 patients with CLL stages IIB–III were included in the study (Tables I and II) NHL was classified histopathologically according to Rappaport et al (16) Favourable histologies were non histiocytic nodular lymphomata plus diffuse lymphocytic well differentiated unfavourable histologies were histiocytic and diffuse lymphocytic poorly differentiated and diffuse mixed lymphomata Clinical stage was determined according to the Ann Arbor classification (4) All patients with CLL fulfilled the generally accepted criteria and were staged according to guidelines from CALGB (17)

All patients had previously received alkylating agents and prednisone in various regimens Treatment had however been discontinued in 14 patients with NHL and in 5 with CLL due to progressive disease and therefore probably clinical resistance to the drugs (Tables I and II) All

*Abbreviations* CLL chronic lymphocytic leukaemia NHL non Hodgkin lymphomata CR = complete remission PR partial remission IMP improvement

Table I *Clinical characteristics and results of treatment with Prednimustine in 16 patients with previously treated NHL*

| Pat no | Age (y) | Sex | Histological diagnosis | Stage | Previous cytostatic treatment | Disease progressiveness by previous therapy | Prednimustine: No of 5 day courses with 200 mg | Prednimustine: No of days with 60 mg initially | Response: Objective | Response: Duration (weeks) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 30 | ♂ | NLWD | IIIB | VCR + Pred + Stn<br>Ctx bolus + VCR + Pred<br>CAVOP | No | | 153 | PR | 13 |
| 2 | 65 | ♀ | NLWD | IVB | VCR+Pred Ctx bolus<br>CLB CAVOP | Yes | | 14 | NC | |
| 3 | 73 | ♀ | NLPD | IIIA | VCR + Pred + Stn<br>Ctx bolus + VCR + Pred | Yes | | 200 | CR | 69 |
| 4 | 50 | ♀ | NLPD | IVB | VCR + Pred + Stn<br>Ctx bolus + VCR + Pred<br>CAVOP | Yes | | 98 | CR | 39 |
| 5 | 45 | ♂ | NLPD | IVB | VCR + Pred + Ctx p o<br>CAVOP | Yes | 4 | 111 | PR | |
| 6 | 39 | ♀ | NLPD | IIIB | VCR + Pred + Ctx p o<br>Bleo Cis Plat | Yes | | 14 | NC | |
| 7 | 70 | ♂ | NM | IVB | VCR + Pred Ctx p o<br>VP 16 | Yes | 4 | | PR | 9 |
| 8 | 67 | ♂ | DLWD | IVB | CLB + Pred<br>VCR + Pred + Ctx p o<br>VP 16 + Pred Adm | Yes | 1 | | NC | |
| 9 | 67 | ♂ | DLPD | IVA | CAVOP | Yes | | 165 | PR | 30 |
| 10 | 31 | ♂ | DLPD | IVA | CAVOP Ctx bolus + Pred | No | 2 | | NC | |
| 11 | 61 | ♀ | DM | IVB | VCR+Pred+Ctx bolus<br>CAVOP VCR + Pred+<br>Ctx bolus | Yes | | 65 | PR | 5 |
| 12 | 60 | ♀ | DH | IVB | CVPP Ctx p o →<br>bolus CAVOP | Yes | 1 | | PR | 9 |
| 13 | 61 | ♀ | DH | IVA | VCR + Pred + Stn<br>Ctx bolus + VCR + Pred<br>CAVOP | Yes | 4 | | NC | |
| 14 | 71 | ♂ | DH | IVB | VP 16 VCR + Pred + Ctx<br>p o Adm | Yes | 1 | | NC | |
| 15 | 71 | ♀ | DH | IIIB | VCR + Pred Ctx p o<br>CAVOP | Yes | | 21 | NC | |
| 16 | 54 | ♀ | DH | IVB | CAVOP Adm | Yes | | 19 | PD | |

NLWD = nodular lymphocytic well differentiated NLPD = nodular lymphocytic poorly differentiated NM = nodular mixed DLWD = diffuse lymphocytic well differentiated DLPD = diffuse lymphocytic poorly differentiated DM = diffuse mixed DH = diffuse histiocytic VCR = vincristine Pred = prednisone Stn = streptonigrin Ctx = cyclophosphamide CLB = chlorambucil Bleo = bleomycin Adm = adriamycin VP 16 = demethylepipodophyllotoxin-ethylidine glucopyranoside Cis Plat = cis platinum diamine dichloride CAVOP = Ctx + Adm + VP 16 + VCR + Pred CVPP = chlorethyl cyclohexyl nitrosourea + vinblastine + procarbazine + Pred CR = complete remission PR = partial remission IMP = improvement NC = no change PD = progressive disease

patients had at least two measurable parameters at the institution of Prednimustine treatment No chemotherapy or radiotherapy had been given for at least 2 weeks before the start of Prednimustine no nitrosourea preparations for 6 weeks Initially a complete physical examination was carried out with laboratory tests including Hb leucocyte differential and platelet counts reticulocytes serum creatinine uric acid bilirubin SGOT alkaline phosphatase and urine analyses In all patients bone marrow biopsy from the iliac crest had been performed and in patients with lymphomata relevant radiological studies comprising chest radiography and lymphangiograms These parameters were followed regularly during treatment

Prednimustine tablets 20 and 100 mg were supplied by Leo Halsingborg Sweden Two schedules were used 1) 80 mg p o daily continuously with stepwise reduction of the dose according to leucocyte and platelet counts and 2) five day courses of 200 mg daily p o every third week with subsequent reduction of the dose according to haematological parameters Patients were treated with at least one course of Prednimustine or continuously for at

| Toxicity | | | | | | | |
|---|---|---|---|---|---|---|---|
| Hb (mmol/l) | | Leucocytes (×10 /l) | | Platelets ($\times 10^9$/l) | | Weeks from start to nadir | Weeks of duration |
| Initial | Nadir | Initial | Nadir | Initial | Nadir | | |
| 7 3 | 7 9 | 5 5 | 1 0 | 239 | 47 | 27 | 5 |
| 7 0 | | 5 2 | | 305 | | | |
| 8 3 | 4 3 | 4 3 | 0 4 | 219 | 20 | 10 | 4 |
| 6 8 | 5 3 | 2 5 | 0 4 | 138 | 43 | 4 | 4 |
| 8 0 | 8 2 | 4 3 | 3 2 | 134 | 94 | 5 | 2 |
| 5 4 | 5 5 | 1 4 | 1 4 | 22 | 19 | | |
| 6 9 | 6 4 | 8 0 | 1 6 | 423 | 41 | 5 | 4 |
| 7 8 | 7 8 | 3 9 | 2 0 | 150 | 100 | 2 | 2+ |
| 6 5 | 6 0 | 4 9 | 1 2 | 173 | 106 | 7 | 4 |
| 8 2 | 8 1 | 7 9 | 2 5 | 187 | 116 | 2 | 3 |
| 6 9 | 6 0 | 4 8 | 1 0 | 127 | 14 | 9 | 2 |
| 6 8 | 6 2 | 3 1 | 1 5 | 160 | 52 | 3 | 4 |
| 7 4 | 7 7 | 5 2 | 2 0 | 275 | 31 | 9 | 4 |
| 8 2 | 7 3 | 3 9 | 3 9 | 304 | 256 | | |
| 7 0 | 6 8 | 4 3 | 1 5 | 129 | 39 | 1 | 1+ |
| 6 5 | | 4 2 | | 738 | | | |

least 14 days One patient did not fulfil this requirement as treatment had to be discontinued after 4 days because of severe side-effects Patients with NHL were treated according to both schedules patients with CLL were all treated with regimen I

Complete remission (CR) was defined as complete disappearance of all measurable parameters of disease partial remission (PR) as a reduction of tumour size ($cm^2$)—and in CLL also of lymphocyte counts by more than 50% *Improvement* (IMP) was defined as a reduction of up to 50% of measurable parameters

## RESULTS

Remission was obtained in 8/16 patients with NHL and in 6/14 patients with CLL In addition 5 patients with CLL showed good improvement

Of the patients with NHL (Table I) two obtained a CR of 39+ and 69+ weeks duration 6 had a PR Median duration of response for lymphomata was 12 weeks Histology seemed of importance for response thus 5/8 patients with favourable histology responded (2 CR) versus 3/8 patients with unfavourable histology (all PR) In the latter group a long lasting response was obtained in only one patient with diffuse lymphocytic poorly differentiated lymphoma Schedule of Prednimustine age sex stage of the disease and type of previous therapy were not of importance for response in this study It was noteworthy that 7/8 remissions in NHL were obtained in patients who had progressive disease during the previous therapy with cyclophosphamide and prednisone Doses of cyclophosphamide in these patients had varied between 500 och 1 600 mg given as i v push in 5 while two patients had received cyclophosphamide 100 and 150 mg daily p o Doses of prednisone had varied between 40 and 70 mg daily All patients had been treated for at least two months and four had marked haematological toxicity during therapy which prevented further increase in dosage

Of the patients with CLL (Table II) all but two (*nos 1 and 13*) *responded with a marked decrease in* the lymphocyte count in peripheral blood and 12/14 responded with a redution in size of lymph nodes and/or the spleen However CR was not obtained Median duration of response in CLL was more than 15 weeks with 7/11 responding patients still in remission at the time of evaluation Five of the CLL patients had progressive disease on previous therapy including chlorambucil and prednisone and 3 responded one with PR two with IMP The previous regimen in these 3 patients was chlorambucil 4–6 mg daily p o given for at least two months and prednisone 10–40 mg daily At least one of the patients had marked haematological toxicity during treatment with chlorambucil which prevented further increase in dosage

Toxicity following treatment with prednimustine was registered as leucopenia and/or thrombocytopenia in 25/30 patients Two patients with NHL (nos 2 and 16) could not be evaluated in this respect because of an unavoidable abrupt shift to

Table II *Clinical characteristics and results of treatment with Prednimustine in 14 patients with previously treated CLL*

| Pat. no | Age (y) | Sex | Stage | Previous cytostatic treatment[a] | Disease progression by previous therapy | Response: Leucocytes ×10⁹/l/ % lymphocytes p b — Initial | Nadir | Reduction in size of lymph nodes or spleen[b] | Overall response[a] | Duration (weeks) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 77 | ♀ | IIB | Pred + CLB | Yes | 9.5 83 | 35 8/99 | + | NC | |
| 2 | 57 | ♂ | IIB | Pred CLB<br>Pred + CLB | Yes | 27 2/90 | 4 3/53 | ++ | PR | 6 |
| 3 | 67 | ♀ | IIB | Pred<br>Pred + CLB | No | 20 7 80 | 3 0/43 | ++ | PR | 16 |
| 4 | 65 | ♂ | IIB | Pred<br>Pred + CLB | No | 36 4/87 | 0 8/46 | ++ | PR | 15 |
| 5 | 54 | ♂ | IIB | Pred + CLB | No | 80 0/93 | 20 4/97 | + | IMP | 4 |
| 6 | 57 | ♂ | III A | CLB<br>Pred + CLB | No | 45 5/95 | 3 0,82 | ++ | PR | 9 |
| 7 | 71 | ♂ | IIB | Pred + CLB<br>CLB | Yes | 20 0 86 | [illegible] 8,64 | + | IMP | 16 |
| 8 | 34 | ♂ | IIB | Pred + CLB | No | 16 3/43 | 0 8/97 | + | IMP | 27 |
| 9 | 70 | ♀ | IIB | VCR + Pred +<br>VP 16 CLB | No | 17 4/80 | 4.2/47 | ++ | PR | 18 |
| 10 | 68 | ♂ | IIB | CLB | No | 120/99 | 7 1/52 | ++ | PR | 16 |
| 11 | 69 | ♂ | IIB | Pred + CLB | No | 44 0/93 | 7 2/93 | 0 | NC | |
| 12 | 65 | ♀ | IIB | VCR<br>Pred + CLB | Yes | 3 8/77 | 1 2/37 | 0 | NC | |
| 13 | 75 | ♂ | IIB | VCR + Pred<br>Ctx p o<br>CLB | No | 4 0/27 | 4 2/9 | + | IMP | 12 |
| 14 | 64 | ♂ | III A | Pred + CLB<br>CAVOP | Yes | 4 9/59 | 1 4/22 | + | IMP | 6 |

[a] Abbreviations as in Table I
[b] ++ = 50–100% reduction in size + 0–50% reduction in size 0 no change

other therapy and one patient (no 6) because of lymphoma infiltration of the marrow In two patients one with NHL (no 14) and one with CLL (no 13) no toxicity was observed

The grade of haematological toxicity in patients with normal values of Hb leucocytes and platelets at the start of Prednimustine therapy is shown in Table III The toxicity which as shown could be severe was maximal at 1 27 weeks (median 6) from the start of Prednimustine with a duration of 1–8 weeks (median 4) No significant difference in toxicity was observed between the two schedules used 80 mg p o daily and courses of 200 mg p o daily for five days However only 7 patients with NHL were treated with the latter regimen

Four patients with NHL complained of dyspeptic symptoms and in three of these severe complications developed In two cases intestinal perforation

Table III *Haematological toxicity of Prednimustine in patients with normal parameters at the initial examination*

| Prednimustine dosage | Anaemia (mmol/l)[a] 0 | I | II | III | IV | Leucopenia (×10⁹/l)[b] 0 | I | II | III | IV | Thrombocytopenia (×10⁹/l)[c] 0 | I | II | III | IV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 200 mg×5 | [illegible] | 1 | | | | | | [illegible] | 1 | | 1 | 2 | 1 | 2 | |
| 80 mg/d | 3 | 5 | 1 | 3 | | 1 | 3 | 1 | 5 | 3 | [illegible] | 4 | 3 | 2 | 1 |

[a] I 6.2– 4 II = 5.9–6.1 III = 4.3–5.8 IV = <4.3
[b] I 3–3.9 II [illegible] 2.9 III = 1 1.9 IV = <1.0
[c] I = 100–150 II = 50–99 III = 20–49 IV <20

Toxi ty

| Hb (mmol/l) | | Platelets (×10⁹/l) | | Weeks from start to nadir | Weeks of du ration |
|---|---|---|---|---|---|
| Initial | Nadir | Initial | Nadir | | |
| 8 6 | 8 2 | 124 | 72 | 10 | 2 |
| 8 2 | 6 6 | 168 | 87 | 6 | 4 |
| | 6 1 | 80 | 57 | 4 | 6 |
| 8 9 | 5 1 | 282 | 65 | 15 | 4 |
| 10 3 | 7 0 | 213 | 6 | 6 | 8 |
| 6 9 | 6 5 | 119 | 105 | 5 | 4 |
| 7 9 | 6 8 | 216 | 131 | 2 | 1 |
| 8 4 | 4 6 | 187 | 138 | 4 | 2 |
| 7 0 | 6 8 | 184 | 162 | 7 | 2 |
| 8 0 | 7 4 | 247 | 59 | 16 | 4 |
| 8 1 | 5 9 | 106 | 35 | 6 | 2 |
| 8 8 | 6 9 | 172 | 109 | 7 | 2 |
| 8 9 | 9 2 | 276 | 195 | | |
| 5 1 | 4 8 | 120 | 146 | 4 | 2 |

due to lymphoma infiltration of the small intestine was seen and in one patient haematemesis of un known origin occurred In the latter patient Pred nimustine therapy was resumed later without symptoms from the gastrointestinal tract In one patient with NHL (not included among the patients evaluable for response see Patients and Methods) Prednimustine was discontinued after four days treatment with 80 mg daily p o owing to dyspeptic symptoms and a severe psychosis like reaction

## DISCUSSION

Prednimustine is an interesting new compound synthesized from two drugs with well known cyto-static activity The pharmacological data the clinical characteristics (limited toxicity at a daily dose of 80 mg p o equivalent to around 40 mg chlorambucil and 40 mg prednisolone) and the therapeutic effect in patients clinically resistant to alkylating agents and prednisone as demonstrated in some patients in the present study clearly indicate that Pred nimustine is not merely a simple substitute for the two components prednisolone and chlorambucil

So far the most promising results have been ob-tained in lymphoid neoplasias a 75% response rate both in patients with CLL (3 5 8 and present study) and in NHL of lymphocytic type (Table IV) This response frequency is remarkably high in view of the fact that many patients had received exten sive prior treatment including alkylating agents

Several dose levels and schedules of administra tion have been used in the published series One study (6) seems to indicate a dose–response rela tionship in NHL but a definite conclusion as to the optimal dose and schedule must await larger com parative studies A major problem with intensive Prednimustine treatment is the late and rather ab-ruptly appearing myelosuppression of prolonged duration This problem can probably be avoided if Prednimustine is used in lower doses in combina tion chemotherapy with other drugs this has still to be defined

We find the antineoplastic activity of this new drug so encouraging that larger phase III studies in lymphoid neoplasias should be undertaken espe cially with the intention of exploring its use in com bination chemotherapy

Table IV *Results of Prednimustine treatment in 93 patients with NHL subclassified according to Rappaport*

Data are compiled from ref 6 13 14 and our own series

| | N | CR | PR | NR |
|---|---|---|---|---|
| Rappaport favourable[a] | | | | |
| Previously untreated | 23 | 10 | 10 | 3 |
| Previously treated[c] | 19 | 5 | 9 | 5 |
| Treatment unknown | 4 | 1 | 2 | 1 |
| Total | 46 | 16 | 21 | 9 |
| Rappaport unfavourable[b] | | | | |
| Previously untreated | 13 | 7 | 4 | 2 |
| Previously treated | 29 | 9 | 9 | 11 |
| Treatment unknown | 5 | 1 | 3 | 1 |
| Total[d] | 47 | 17 | 16 | 14 |

[a] Nodular lymphocytic well differentiated nodular lym phocytic poorly differentiated nodular mixed diffuse lymphocytic well differentiated

[b] Diffuse lymphocytic poorly differentiated diffuse mixed diffuse histiocytic nodular histiocytic

[c] In the majority of cases previous treatment included alkylating agents and prednisone

[d] Diffuse lymphocytic poorly differentiated in 34 diffuse histiocytic in only 9

## REFERENCES


1 Brandt L & Konyves I Therapeutic effect of Prednimustine (Leo 1031) in various types of leukaemia Eur J Cancer 13 393 1977
2 — Use of Prednimustine for remission induction without drug induced bone marrow aplasia in adult acute non lymphocytic leukaemia (ANLL) Med Oncology 3 521 1978
3 Brandt L Konyves I & Moller T R Therapeutic effect of Leo 1031 an alkylating corticosteroid ester in lymphoproliferative disorders I Chronic lymphocytic leukaemia Acta Med Scand 197 317 1975
4 Carbone P P Kaplan H S Musshoff K Smithers D W & Tubiana M Report of the committee on Hodgkin s disease staging classification Cancer Res 31 1860 1971
5 Clinical Screening Cooperative Group of E O R T C A phase II clinical trial of Prednimustine Biomed 27 158 1977
6 Håkansson L Konyves I Lindberg L G & Moller T Continuous treatment of non Hodgkin s malignant lymphoma with Prednimustine (Leo 1031) Oncology 35 103 1978
7 Internal research communication Leo Pharmaceuticals Halsingborg Sweden Feb 1977
8 Kaufman J H Hanjura G L Mittelman A Aungst C W & Murphy G P Study of Leo-1031 (NSC 134087) in lymphocytic lymphoma and chronic lymphocytic leukemia Cancer Treat Rep 60 277 1976
9 Kirdani R Y Murphy G P & Sandberg A A Some metabolic aspects of a nitrogen mustard of prednisolone Oncology 35 47 1978
10 Konyves I & Liljekvist J The steroid molecule as a carrier of cytotoxic groups Excerpta Med Int Congr Ser 375 98 1975
11 Konyves I Nordenskjold B Forshell G P De Schryver A & Westerberg Larsson H Preliminary clinical and absorption studies with Prednimustine in patients with mammary carcinoma Eur J Cancer 11 841 1975
12 Lele S B Piver M S Barlow J & Murphy G P Leo 1031 (NSC 134087) in gynecological malignancies Oncology 35 101 1978
13 Mattsson W von Eyben F Turesson I & Wahlby S Prednimustine (NSC 134087 Leo 1031) treatment of lymphocytic and lymphocytic histiocytic lymphomas Cancer 41 112 1978
14 Moller T R Brandt L Konyves I & Lindberg L G Therapeutic effect of Leo 1031 an alkylating corticosteroid ester in lymphoproliferative disorders II Lymphocytic lymphoma Acta Med Scand 197 323 1975
15 Mouridsen H T Kristensen D Nielsen J I & Dombernowsky P Phase II trial of Prednimustine L 1031 (NSC 134087) in advanced breast cancer Cancer In press 1979
16 Rappaport H Winter W J & Hicks H B Follicular lymphoma a re evaluation of its position in the scheme of malignant lymphomas based on a survey of 253 cases Cancer 9 792 1956
17 Silver R T Sawitsky A Rai K Holland J F & Glidewell O Guidelines for protocol studies in chronic lymphocytic leukemia Am J Hematol 4 343 1978
18 Wilkinson R Gunnarsson P O Plym Forshell G Renshaw J & Harrap K R The hydrolysis of Prednimustine by enzymes from normal and tumor tissues Excerpta Med Int Congr Ser 420 260 1978

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# Treatment of Osteoporosis with 1-Alpha-Hydroxycholecalciferol and Calcium

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ABSTRACT A double-blind comparative study of 1α OHD3 and placebo was performed on 37 patients with osteoporotic hip fracture without clinical osteomalacia 1α OHD3 in a dose of 1 μg/day together with 2 5 g $CaCO_3$, did not heal osteoporosis as judged from determinations of bone mineral density and histomorphometric analyses during four months of treatment However 1α OHD3 seemed to have an effect on fracture healing as concluded from the posttreatment alkaline phosphatase level Hypercalcemia was common, occurring in six out of 19 patients treated with 1α OHD3 It is concluded that treatment of osteoporosis with 1α OHD3 and calcium is ineffective and potentially dangerous because it frequently causes hypercalcemia

*Key words* osteoporosis hip fracture cholecalciferol
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1 Alpha hydroxycholecalciferol (1α OHD3) is a synthetic analogue of vitamin D which is converted to its active metabolite 1 25-dihydroxy cholecalciferol by the liver 1α OHD3 has been used in the treatment of osteoporosis with varying success (12 14 15) whereas in most studies calcium therapy had no effect on bone mineral density (BMD) in osteoporosis (9)

We carried out a controlled clinical trial to assess the combined effect of 1α OHD3 and calcium on bone mineral content muscle strength and histomorphometric values in patients with osteoporotic hip fracture

## PATIENTS AND METHODS

Thirty seven consecutive patients who were admitted to University Central Hospital in Kuopio for a hip fracture caused by moderate or no trauma were included in the study All patients were operated on for the fracture Patients younger than 50 years of age those with poor cooperation or signs of renal disease were excluded Moreover two patients with clinically evident osteomalacia by biochemical and roentgenologic criteria (hypocalcemia elevated serum alkaline phosphatase (S-ALP) and Milkman Looser pseudofractures) were excluded

The patients were divided into two groups The first group was treated daily with 1 μg of 1α-OHD3 and 2 5 g of $CaCO_3$ (Ca ~ 1 0 g) for four months There were 19 patients in this group 14 women and 5 men with a mean age of 73 years (range 60–86) The second group given placebo and 2 5 g $CaCO_3$ comprised 18 patients 15 women and 3 men with a mean age of 75 years (range 55–84)

S-ALP creatinine total protein and the urinary calcium/creatinine ratio from a single sample were determined by routine laboratory methods at the beginning of the study after three months and finally after six months of treatment Serum calcium was monitored at 3-week intervals

BMD (g/cm²) was determined by the Am 241 gamma ray attenuation method at the distal radius of the patient s non-dominant forearm (8) Control values were obtained from an age and sex matched group of healthy persons from a previous study (2)

The muscle strength (kp/cm²) was measured from both hands using the Martin vigorimeter The result was presented as the mean of three measurements BMD and muscle strength were evaluated on three occasions on admission to the study after three months and after six months of treatment

Histomorphometric values were studied at the beginning and after six months of treatment Iliac bone biopsies were performed for histomorphometric evaluation The first biopsy was taken from the anterior part of the iliac crest on the fracture side The second biopsy was made on the opposite side Undecalcified methacrylate-embedded 5 μm thick bone sections were cut using a motor-driven Jung microtome The sections were stained with Masson Goldner trichrome stain The histomorphometric analysis was performed blindly according to Merz (13) The volumetric density of trabecular bone ($V_v$) trabecular

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*Abbreviations* 1α-OHD3 1 alpha hydroxycholecalciferol S-ALP = serum alkaline phosphatase $V_v$ = volumetric density of trabecular bone $V_{vo}$ = volumetric density of osteoid seams OS% = trabecular bone surface covered with osteoid BMD = bone mineral density

Table I *Bone mineral density and muscle strength of the non dominant forearm and S ALP values in patients treated with 1α OHD3 or placebo (mean±1 S D )*

| | 1α OHD3 group ($n$=19) | Placebo group ($n$=18) |
|---|---|---|
| *BMD (g/cm²)* | | |
| Before treatment | 0 233±0 019 | 0 231±0 029 |
| After 3 months of treatment | 0 229±0 018 | 0 235±0 023 |
| After 6 months of treatment | 0 236±0 017 | 0 231±0 021 |
| *Muscle strength (kp/cm²)* | | |
| Before treatment | 0 41±0 25 ($n$=18) | 0 43±0 23 ($n$=16) |
| After 3 months of treatment | 0 46±0 21 ($n$=16) | 0 46±0 24 |
| After 6 months of treatment | 0 45±0 17 ($n$=13) | 0 48±0 25 ($n$=15) |
| *S ALP (IU/l)* | | |
| Before treatment | 175±60 ($n$=18) } n s | 166±48 } $p$<0 001 |
| After 3 months of treatment | 189±56 ($n$=18) | 243±61 |

bone surface covered with osteoid (OS%) and the percentage of trabecular bone volume occupied by osteoid ($V_{vo}$) were determined According to Woods et al (16) the upper normal limit for osteoid volume is 5% and for OS% 45% An iliac bone biopsy was made on every patient but the histological sections made of these bone biopsy specimens were not adequate in every case for histomorphometric analysis Histomorphometric values were measured from 27 patients before treatment (11 from the 1α OHD3 and 16 from the placebo group) and from 13 patients after treatment (6 from the 1α OHD3 and 7 from the placebo group) In 10 patients (3 from the 1α-OHD3 and 7 from the placebo group) it was possible to carry out a histomorphometric analysis both before and after six months of treatment

Student s $t$ test for paired observation was used in the statistical analysis

## RESULTS

The mineral density of the distal radius was slightly lower in both patient groups than in healthy controls but the difference was not statistically significant The treatment did not change the BMD or the muscular force in either group (Table I)

S ALP values were within normal limits in both groups immediately after the trauma The mean value was statistically significantly increased after three months of treatment in the placebo group but not in the 1α OHD3 group (Table I) The urinary calcium/creatinine ratio increased from 0 39±0 29 to 0 73±0 33 ($p$<0 005) in the 1α OHD3 group and from 0 37±0 36 to 0 48±0 40 (n s ) in the placebo group after three months of treatment The mean serum calcium level increased from 2 33±1 35 mmol/l to 2 48±1 61 mmol/l ($p$<0 001) in the 1α OHD3 group but there was no change in the placebo group after three months of treatment

In the histomorphometric study before treatment six patients out of 36 showed a slightly increased $V_{vo}$ or OS% No statistically significant changes were found in the mean $V_v$ or in the extent of osteoid seams before and after treatment in either group but there was a decrease ($p$<0 05) in $V_{vo}$ in the 1α OHD3 group after treatment (Table II)

### Side effects

Hypercalcemia was found in eight patients during the treatment six in the 1α OHD3 group and two in

Table II *Histomorphometric values (mean±1 S D ) before (I) and after six months of treatment (II) with 1α-OHD3 or placebo*

| | 1α OHD3 group | | Placebo group | |
|---|---|---|---|---|
| | I | II | I | II |
| $V_v$ (%) | 15 8± 4 8 | 13 8±4 3 | 14 4± 4 6 | 13 7±6 6 |
| OS% | 19 5±12 7 | 16 5±4 7 | 18 7±14 0 | 15 9±8 4 |
| $V_{vo}$ | 3 0±4 0 | 0 4±0 4 | 2 4± 2 0 | 1 1±1 6 |

the placebo group Two cases of hypercalcemia in the 1α OHD3 group were so severe that the patients had to be hospitalized and the drug withdrawn

## DISCUSSION

Femoral neck fracture is a frequent complication of osteoporosis Some patients with a hip fracture also have osteomalacia in addition to osteoporosis (1 5 6 7) 1α OHD3 is a potent vitamin D analogue that heals osteomalacia (3) It has been postulated that 1α OHD3 is capable of increasing bone mass also in osteoporotic patients Lindholm et al (12) demonstrated an increase in bone mineral content in two out of five senile and three out of five postmenopausal osteoporotic patients during long term treatment with 1α OHD3 The other patients showed a non significant decrease in bone mineral content Sørensen et al (15) showed an increase in bone mineral content in their 26 osteoporotic patients after treatment with 1α OHD3 2 μg/day but no effect was observed with a smaller dose 1 μg/day This was probably due to the improvement in the histologically evident osteomalacic changes that were found in one third of their patients Neither was there any change in the total bone area of trabecular bone

We evaluated the effect of 1α OHD3 treatment on patients with osteoporotic hip fracture Patients with clinically evident osteomalacia were excluded Histological osteomalacia was observed in six out of 36 cases according to criteria proposed by Woods et al (16) However this slight osteomalacia is a minor factor compared with osteoporosis for bone strength (5)

In the present series treatment with 1α OHD3 did not increase the BMD measured by the Am 241 gamma ray attenuation method Neither did 1α OHD3 increase the muscular force during treatment

In the histomorphometric studies the amount of osteoid seemed to decrease in the 1α-OHD3 group during treatment but V did not change which is in accordance with our results using photon absorptiometry

The serum calcium level and urinary excretion of calcium increased in the 1α OHD3 but not in the placebo group In the 1α OHD3 group S-ALP level was not increased but in the placebo group it was higher after three months of treatment than before This is probably due to the effect of vitamin D on fracture healing (11)

In our series 1α OHD3 increased calcium absorption had possibly a small effect on fracture callus formation and improved histological osteomalacia but did not heal osteoporosis Our observations on the effect of 1α OHD3 treatment on osteoporosis are similar to those presented by Buring et al (4) who used vitamin D2 Moreover even the small dose of 1α OHD3 which was used in the present study had a strong hypercalcemic effect as was also demonstrated by Sørensen et al (15) This will probably hamper the use of this drug in clinical practice

Why did two of our patients in the placebo group as well have temporary hypercalcemia? One explanation is that they were old and their hydration was insufficient They might also have had mild renal insufficiency because a normal serum creatinine level does not exclude mild impairment of renal function It is known that calcium carbonate in large doses may cause hypercalcemia (10) but in our study the dose of $CaCO_3$ was relatively small

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## REFERENCES

1 Aaron J E Gallagher J C Anderson J Stasiak L Longton E B Nordin B E C & Nicholson M Frequency of osteomalacia and osteoporosis in fractures of the proximal femur Lancet 1 229 1974
2 Alhava E M & Karjalainen P The mineral content and mineral density of bone of the forearms in healthy persons measured by Am 241 gamma ray attenuation method Ann Clin Res 5 238 1973
3 Bordier P Pechet M Hesse R Marie P & Rasmussen H Response of adult patients with osteomalacia to treatment with crystalline 1α hydroxy vitamin $D_3$ N Engl J Med 291 866 1974
4 Buring K Hulth A Nilsson B E Westlin N E & Wiklund P E Treatment of osteoporosis with vitamin D Acta Med Scand 195 471 1974
5 Chalmers J Barclay A Davison A M Macleod D A D & Williams D A Quantitative measurement of osteoid in health and disease Clin Orthop 63 196 1969
6 Eid A M Osteomalacia as a contributing factor in fracture of the femoral neck in the elderly in Qatar Clin Orthop 132 129 1978
7 Gallagher J C Aaron J Nicholson M Longton E & Nordin B E C The role of osteoporosis and

Table I *Prevalence of dyspnea (%)*

| | Males (n=446) | Females (n=518) | p |
|---|---|---|---|
| Subjective dyspnea only | 13 0 | 23 6 | <0 001 |
| Tachypnea only | 15 0 | 9 5 | <0 01 |
| Subjective dyspnea and tachypnea | 17 5 | 12 5 | <0 05 |
| Total | 45 5 | 45 6 | N S |

consumption The medical examination included a thorough medical examination ECG recordings that were coded according to the Minnesota code (11) and a roentgenological examination of the chest Heart volume was determined according to the method of Jonsell (3)

The probands were considered to fulfill the criteria for dyspnea if the examining physician found the proband to be tachypnoic at rest or when undressing or if the proband answered yes to the question Do you feel a shortness of breath when walking two stairs or the equivalent as fast as other persons of your own age?

Pulmonary disease was defined as one or more of the following findings chronic bronchitis according to WHO (1) an anamnestic statement of asthmatic bronchitis during the last 10 years emphysema as judged by roentgenological examination and/or decreased heart sounds *plus* decreased respiratory sounds rhonchi at the medical examination roentgenological evidence of bronchopneumonia or pulmonary malignancy Lung function was assessed from the peak expiratory flow rate (PEFR) measured with a Wright peak flow meter (13)

The following criteria were used as signs of heart insufficiency roentgenological evidence of increased heart volume plus two of the symptoms cyanosis dyspnea and edema all the three symptoms cyanosis dyspnea and edema roentgenological evidence of pulmonary congestion roentgenological evidence of increased heart volume in the absence of systemic hypertension A heart volume of at least 450 ml/m² BSA in males and 400 ml/m² in females on digitalis treatment and of at least 500 and 450 ml/m² respectively in those without such treament was taken as evidence of cardiac enlargement

Systemic hypertension was defined as a casual diastolic BP of at least 115 mmHg (phase 4 cuff method) or an anamnestic statement of antihypertensive treatment

Angina pectoris and/or probable ECG evidence of myocardial ischemia as defined by Rose (9 10) were used as evidence of ischemic heart disease (IHD)

Probands who regularly smoked 1 cigarrette or more per day were recognized as smokers and those who had never smoked regularly during their life as non smokers

The interview included questions concerning previous profession the nature of work as well as interest in e g physical activity during leisure time

The statistical methods used were Student s $t$ test and $\chi^2$ tests

## RESULTS

According to our definition 45 5% of the males and 45 6% of the females were dyspnoic i e there was no sex difference (Table I) However signficantly more women had an anamnestic statement of breathlessness during exercise 23 6% of the females and 13 0% of the males had a subjective dyspnea without any signs of respiratory dysfunction at the medical examination ($p<0\ 001$) 15 0% of the males and 9 5% of the females were tachypnoic at the medical examination without an anamnestic dyspnea ($p<0\ 01$)

*Of the dyspnoic males and females 33 0 and* 35 6% respectively fulfilled our criteria of cardiac failure a prevalence significantly different from that in probands without dyspnea ($p<0\ 001$) (Table II) 44 3% of the males and 19 5% of the females with dyspnea had at least one of the signs of pulmo-

Table II *Prevalence of cardiac failure and pulmonary disease (%)*

| | Males | | Females | |
|---|---|---|---|---|
| | With dyspnea (n=203) | Without dyspnea (n=243) | With dyspnea (n=236) | Without dyspnea (n=282) |
| Cardiac failure | 33 0 | 19 3*** | 35 6 | 12 8*** |
| Pulmonary disease | 44 3 | 23 5 * | 19 5 | 13 1* |
| Bronchitis WHO | 23 6 | 12 8* | 10 2 | 8 5 |
| Asthmatic bronchitis | 4 9 | 0 1 | 5 9 | 1 1 * |
| Rhonchi | 18 7 | 5 3 ** | 8 1 | 1 8 ** |
| Emphysema X ray | 12 8 | 5 8 | 2 1 | 0 4 |
| Emphysema physical examination | 13 3 | 5 3 | 0 4 | 0 7 |
| Malignancy | 0 | 1 2 | 0 | 0 |
| Bronchopneumonia | 2 5 | 0 8 | 0 8 | 0 7 |
| No cardiac failure or pulmonary disease | 35 5 | 62 1* | 52 1 | 76 2*** |

* $p<0\ 05$ * $p<0\ 01$ $p<0\ 001$ ($\chi^2$ test)

Table III. *Pulmonary findings in probands without cardiac failure and pulmonary disease (%)*

| | Males | | Females | |
|---|---|---|---|---|
| | With dyspnea ($n=72$) | Without dyspnea ($n=151$) | With dyspnea ($n=123$) | Without dyspnea ($n=214$) |
| Irregular sputal production | 26.4 | 16.6 | 14.6 | 11.7 |
| Rales | 13.9 | 6.6 | 10.6 | 5.6 |
| Inactive roentgenological findings | 48.6 | 41.1 | 39.8 | 30.4 |

nary disease listed in Table II. The difference from probands without dyspnea was statistically significant at the 0.1% level for males but not at the 1%-level for females. Chronic bronchitis according to WHO definition was the most common pulmonary disease. 35.5% of the males and 52.1% of the females with dyspnea had neither cardiac failure nor pulmonary disease *compared to 62.1% and* 76.2% of males and females without dyspnea.

Among probands without cardiac failure and pulmonary disease there was no statistically significant difference between probands with dyspnea and others with regard to irregular sputal production, rales at the medical examination or inactive roentgenological pulmonary findings (Table III).

Among probands without cardiac failure or pulmonary disease 19.5% of the females with dyspnea and 8.4% of other females had been inactive in their leisure time earlier during life ($p<0.01$). Such a difference was not observed in males.

According to the psychiatric evaluation, signs of irritability and/or emotional lability were present in 36% of dyspnoic males but in only 9.7% of non dyspnoic males ($p<0.01$). The corresponding figures for females were 25 and 9.4% ($p<0.01$).

Among probands without cardiac failure or pulmonary disease there was a significantly higher prevalence of IHD in the dyspnoic group. 22.2% of 72 males with and 7.9% of 151 males without dyspnea had IHD ($p<0.01$). Of 123 females with dyspnea 17.1% had IHD compared to 6.1% of 214 females without ($p<0.001$). Probands with disorders statistically correlated to dyspnea, i.e. cardiac failure, IHD and pulmonary disease, have been withdrawn in the further treatment of the material.

The experience of subjective health measured in terms of general fatigue and anamnestic statement of health experience showed that 41.4% of dyspnoic and 17.3% of non-dyspnoic males did not feel healthy ($p<0.001$). In females there was a corresponding tendency, 37.3% and 26.9%, although not statistically significant (Table IV). 28.6% of the dyspnoic males and 33.3% of the dyspnoic females had a general feeling of fatigue, while the corresponding prevalance of fatigue in probands without dyspnea was 7.2% in males and 14.9% in females ($p<0.001$). *There were no differences in social isolation,* drug treatment and/or smoking habits between probands with and without dyspnea (Table IV).

As a general objective health screening we have besides the general clinical examination also examined the relative heart volume, diastolic blood pressure, body weight and some routine laboratory analyses, i.e. B Hb, ESR, S-potassium, S-creatinine, S protein and B glucose. There were no statistically significant differences at the 1%-level between the dyspnoic and non dyspnoic probands at any of these comparisons (Table V) with one exception: males had a significantly larger heart volume if they were dyspnoic ($p<0.01$, Student's *t* test). Both males and females with dyspnea but without cardiopulmonary disease had significantly lower PEFR than others, i.e. 331±95.3 and *370±75.0 l/min in males* ($p<0.01$) *and* 256±62.9 *and* 288±54.3 l/min in females ($p<0.001$) (Table V).

Altogether, among the dyspnoic probands 27.7% of the males and 43.2% of the females had a dyspnea that was not statistically related ($p<0.01$) to detectable disease, i.e. cardiac failure, IHD, pulmonary disease or the above mentioned symptoms. The difference between the sexes was statistically significant ($p<0.01$).

Probands who according to the present definitions of disease were without these disorders have been followed for 5 years after the examination. The mortality rate and causes of death were registered. 16.4% of male probands with and 7.2% without dyspnea died during this period. 10.2% of

Table IV *Observations in probands without cardiac failure IHD and pulmonary disease (%)*

| | Males | | Females | |
|---|---|---|---|---|
| | With dyspnea (n=56) | Without dyspnea (n=139) | With dyspnea (n=102) | Without dyspnea (n=201) |
| Not feeling healthy | 41 1 | 17 3 * | 37 3 | 26 9 |
| Fatigue | 28 6 | 7 2*** | 33 3 | 14 9 ** |
| Social isolation | 17 9 | 10 1 | 24 5 | 22 9 |
| Drug treatment | 64 3 | 50 4 | 67 6 | 74 1 |
| Psychopharmacological drugs | 32 1 | 18 0* | 23 5 | 34 8* |
| Smokers | 41 1 | 43 2 | 10 8 | 10 0 |

* $p<0\ 05$ * * $p<0\ 001$ ($\chi^2$ test)

dyspnoic and 6 5% of non dyspnoic females had died after 5 years The differences were not statistically significant Causes of death according to death certificates are listed in Table VI Only two men and two women died from cardiac failure and no probands died from pulmonary disease

## DISCUSSION

The systematic sample of 70-year olds in our study gave a non response rate of 15% and the comparison between non responders and responders revealed that the responder group was generally representative for the total population of 70-year olds in Gothenburg Sweden (8)

The definition of dyspnea in the present study included probands with tachypnea at the medical examination as well as dyspnea at such an ordinary physical exercise as walking two stairs—a dyspnea that the probands themselves believed to be more pronounced than that of other people of their age Furthermore in the vast majority of cases this subjective dyspnea had appeared or increased in recent years It is therefore of interest to estimate to what extent a subjective experience of dyspnea at the age of 70 is related to disease and whether it is available for treatment The further analysis of the material showed that the prevalence of diseases known to cause dyspnea was higher in this group than in the probands who did not complain of dyspnea No less than 31% of the males and 36% of the females i e one third of the probands complained of exertional dyspnea

The criteria used to define cardiac insufficiency have been thoroughly described elsewhere (5) The further treatment of our material has however shown that our definition of cardiac failure probably leads to overdiagnosis In this definition we used a

Table V *Laboratory results among probands without cardiac failure IHD and pulmonary disease (mean ± S D )*

| | Males | | Females | |
|---|---|---|---|---|
| | With dyspnea (n=56) | Without dyspnea (n=139) | With dyspnea (n=102) | Without dyspnea (n=201) |
| ESR (mm/h) | 13±13 5 | 11±7 9 | 17±11 6 | 14±9 7* |
| B-Hb (g/l) | 150±15 1 | 149±10 5 | 139±11 6 | 140±10 2 |
| S-K (mmol/l) | 3 8±0 26 | 3 8±0 36 | 3 7±0 42 | 3 7±0 49 |
| S-protein (g/l) | 78±6 3 | 78±6 6 | 78±4 6 | 79±4 8 |
| S-creatinine (μmol/l) | 85±18 9 | 86±17 8 | 77±14 1 | 76±19 9 |
| B glucose (mmol/l) | 5 6±1 44 | 5 6±1 65 | 5 6±1 51 | 5 3±0 79 |
| Body weight (kg) | 79±12 0 | 77±10 0 | 69±13 8 | 66±8 7 |
| Diastolic BP (mmHg) | 97±12 6 | 94±11 7 | 98±14 7 | 97±11 3 |
| Heart volume (ml/m²) | 423±59 7 | 398±46 4 | 367±45 6 | 367±44 7 |
| PEFR (l/min) | 331±95 3 | 370±75 0 | 256±62 9 | 288±54 3 |

* $p<0\ 05$ * $p<0\ 01$ $p<0\ 001$ (Student s t test)

Table VI *Five year mortality in probands without cardiac failure IHD and pulmonary disease*

| | Males | | Females | |
|---|---|---|---|---|
| | With dyspnea (*n*=56) | Without dyspnea (*n*=139) | With dyspnea (*n*=102) | Without dyspnea (*n*=201) |
| Overall mortality | 9 | 10 | 10 | 13 |
| Cardiac failure | 0 | 2 | 2 | 0 |
| IHD | 2 | 5 | 3 | 1 |
| Cerebrovascular disease | 2 | 0 | 2 | 3 |
| Malignancy | 4 | 1 | 3 | 7 |
| Other causes | 1 | 2 | 0 | 2 |

limit for normal heart volume that apparently is irrelevant at the age of 70 In the further processing of our material we have not found any significant correlation with symptoms of cardiac failure until the heart volume exceeded 600 ml/m BSA in males and 550 ml/m² in females compared to the values of 500 and 450 ml/m² respectively which are used as upper normal limits in the clinical routine (7)

As far as the respiratory diseases are concerned we consider that with our definition—comprising anamnestic asthmatic bronchitis chronic bronchitis as defined by WHO and emphysema as defined by both roentgenological and clinical findings—we have detected most probands with chronic obstructive pulmonary disease As in the definition of cardiac failure we probably have a certain overdiagnosis of pulmonary disease mainly because of the anamnestic criteria used in the definitions We therefore believe that the percentages of probands with dyspnea but without these diseases known to cause dyspnea at least is not too high

The strong correlation between smoking and obstructive pulmonary disease is well known In patients without clinical evidence of pulmonary disease we did not however find any statistical difference in frequency of dyspnea between smokers and non smokers

As regards probands without cardiac failure or pulmonary disease there was an overrepresentation of IHD among those with dyspnea This correlation has been shown before as has the fact that dyspnea is a stronger risk factor than anginal pain for mortality in coronary heart disease (2 4) Our practical clinical experience indicates that this statement is even more relevant for advanced ages than for upper middle age

Apart from cardiac and pulmonary disease dyspnea was not explained by an increased morbidity in other diseases as far as is illustrated by differences in e g blood pressure and routine laboratory data like B Hb ESR B glucose S-creatinine and S electrolytes Nor was there any difference in total drug consumption between probands with dyspnea and others The only difference was that male probands had a somewhat larger heart volume than other probands Both increased heart volume and dyspnea could obviously depend on a lower elasticity of e g peripheral vessels leading to increased cardiac work load and to a change in respiratory mechanical work Despite these findings probands with dyspnea but lacking definable respiratory or heart disease felt less healthy and were more tired than other probands These probands also had lower PEFR values than other probands This might illustrate a more pronounced ageing of the lungs with e g increased closing volumes pulmonary fibrosis and stiffness of the thorax difficult to diagnose at a medical routine examination but causing dyspnea We had no spirometric examination with which to evaluate any obstruction which of course might be a cause of dyspnea available for treatment Probands with dyspnea but lacking definable pulmonary or circulatory disease suffered significantly more often from irritability and emotional lability—symptoms that are often considered to be a part of a cerebrolesional syndrome—and thus perhaps another sign of a more pronounced ageing

Judging from the mortality figures during the five year observation period it seems obvious that with the criteria used for definable diseases we have not underdiagnosed but may have overdiagnosed

diseases In the group of individuals without definable cardiopulmonary disease the existence of dyspnea was not correlated to any increased mortality rate compared to those without dyspnea and the mortality in cardiopulmonary diseases was low

The females mainly had a subjective dyspnea that could not be verified at the medical examination while the males more often were tachypnoic at the medical examination and more often had definable diseases that apparently caused shortness of breath A general conclusion from our wide population study is that women seem to be more aware than men of different somatic and mental symptoms (12) This might be one reason why they more often than males visited a doctor (12) and also used more drugs (6) A global estimation of the health condition of the two sexes has not indicated that females should suffer from disease more often than male at the age of 70

The present study thus showed that among females with dyspnea only 57% suffered from definable disease known to cause this symptom One possibility is that some of these probands had a pulmonary obstruction impossible to detect at a routine examination but available for treatment if diagnosed with e g spirometry A general finding in our longitudinal retrospective and prospective epidemiological study of elderly individuals is that overdiagnosis is rather common at these ages Further experience from our material will probably indicate that our definitions of diseases also include components of overdiagnosis The figures mentioned here should thus indicate that nearly half of the females and more than a quarter of the males who at this age complain of dyspnea have such a dyspnea as a sign of ageing rather than a symptom of disease

## REFERENCES


1 Fletcher C M Elmes P C Fairbairn A S & Wood C H The significance of respiratory symptoms and the diagnosis of chronic bronchitis in a working population Br Med J 2 257 1959
2 Higgins M W & Keller J B Predictors of mortality in the adult population of Tecumseh Arch Environ Health 21 418 1970
3 Jonsell S A method for the determination of the heart size by teleroentgenography Acta Radiol 20 325 1939
4 Klatsky A L Friedman G D & Siegelaub A B Medical history questions predictive of myocardial infarction J Chronic Dis 29 683, 1976
5 Landahl S Lindblad B Roupe S Steen B & Svanborg A Digitalis therapy in a 70 year old population Acta Med Scand 202 437 1977
6 Landahl S & Steen B Läkemedelskonsumtion hos 70-åringar i Göteborg Läkartidningen 72 5158 1975
7 Landahl S Steen B & Svanborg A Cardiac failure and digitalis therapy in a 70-year-old population 8th European Congress of Clinical Gerontology (Abstract) Neptun Roumania 1977
8 Rinder L Roupe S Steen B & Svanborg A Seventy year-old people in Gothenburg A population study in an industrialized Swedish city I General presentation of the study Acta Med Scand 198 397 1975
9 Rose G A The diagnosis of ischemic heart pain and intermittent claudication in field surveys Bull Org Mond Santé/WHO 27 645 1962
10 — Chest pain questionnaire Milbank Mem Fund Q 43 32 1965
11 The Scandinavian Committee on ECG Classification The Minnesota code for ECG classification Adaptation to CR leads and modification of the code for ECGs recorded during and after exercise Acta Med Scand (Suppl) 481 1967
12 Svanborg A Seventy year old people in Gothenburg. A population study in an industrialized Swedish city II General presentation of social and medical conditions Acta Med Scand (Suppl) 611 5 1977
13 Wright B M & Mc Kerrow C B Maximum forced expiratory flow rate as a measure of ventilatory capacity Br Med J 2 1041 1959

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# Alprenolol-Induced Thrombocytopenia

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**ABSTRACT** A case of alprenolol induced thrombocytopenia in a 65 year old woman is reported She was admitted to the hospital twice with platelet counts below 10×10 l The platelet count rapidly returned to normal after discontinuation of alprenolol The reason for the thrombocytopenia was increased platelet destruction

*Key words* thrombocytopenia alprenolol side-effects of drugs

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Thrombocytopenia has been reported in association with several drugs (1) This side-effect can be caused either by a decreased production or an increased destruction of the platelets Haematological complications during antihypertensive therapy are uncommon One of the best known side effects is thiazide induced thrombocytopenia which is probably caused by suppression of platelet production (1)

During the last ten years the use of $\beta$ blockers in the treatment of hypertension has increased These drugs seem to have very few adverse haematological effects The known pharmacological effect of $\beta$ blockers is an elevation of the platelet level (5) This report concerns an alprenolol induced severe thrombocytopenia with bleeding manifestations

## CASE REPORT

A 65 year-old woman was admitted to the hospital on April 28 because of bleeding manifestations During the last 12 days before admission she had noticed small red spots on her legs and she had had epistaxis and vaginal bleeding for two days

On admission she had petechiae and large bruises on the lower parts of her body Laboratory findings Hb 138 g/l WBC 7 4×10⁹/l with a normal differential count platelet count 1 8×10⁹/l reticulocytes 0 5% ESR 3 mm/h The serum concentrations of creatinine bilirubin alkaline phosphatase aspartate aminotransferase and alanine aminotransferase were normal The tests for factors II VII and X using reagent Simplastin A® and the activated partial thromboplastin time were within the normal range The plasma concentration of fibrinogen was 2 3 g/l The fibrinogen degradation products in serum were not significantly increased Coombs direct and indirect tests were negative and antinuclear antibodies were absent The spleen was of normal size on X ray examination A bone marrow biopsy from the iliac crest showed normal cellularity (30%) and an increased number of megakaryocytes Bone marrow smears were normal

A drug induced thrombocytopenia was suspected on admission and all drugs were therefore discontinued and prednisolone treatment was started Before admission she had been taking the following drugs for several months alprenolol (Aptin® Durules®) bethanidine (Esbatal®) proscillaridin (Talusin®) allopurinol (Zyloric®) and indomethacin (Confortid®) After six days the platelet count was 196×10⁹/l (Fig 1) No antihypertensive treatment was necessary The corticosteroid dose could be gradually reduced (Fig 1) and the patient was discharged after two weeks

At a visit to the Outpatient Clinic on July 26 her blood pressure had increased and alprenolol treatment was started (Aptin® Durules® 0 2 g once daily) At the next visit (Aug 30) she complained of bruises but her platelet count was 1 0×10⁹/l The alprenolol dose was doubled because her hypertension was not adequately controlled On Sept 28 the platelet count had decreased to 18×10⁹/l and so the prednisolone dose was increased (Fig 1) In spite of this the platelet counts continued to decrease and she was admitted to hospital on Oct 9 because of severe thrombocytopenia (8×10⁹/l) and bruises Once again the alprenolol treatment wad discontinued and the platelet counts normalized in 6–7 days Before discharge she was given alprenolol (Aptin® Durules® 0 4 g as a single dose) on one day and in the morning of the next day her platelet count had decreased to 30×10⁹/l She has subsequently been controlled without alprenolol and the prednisolone dose has been gradually decreased The platelet counts have been normal On Feb 13 she was admitted for a provocation test with alprenolol She was given one tablet of Aptin Durules 0 2 g at 10 a m and at 9 p m on the same day The decreases in the platelet counts are shown in Fig 2 Once again the platelet level normalized in a few days

Antihypertensive treatment with propranolol (Inderal® 80 mg twice daily) has subsequently been started without any thrombocytopenia appearing during the first three months The patient has not needed corticosteroid treatment

# Rectal Carcinoma Metastasizing to a Toe

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**ABSTRACT** We report on a 63-year old patient with rectal carcinoma that metastasized to a toe Although bone metastases from malignant tumors are common, metastatic lesions of the small bones of extremities are very rare We have found in the literature only 29 cases of carcinoma which have metastasized to the small bones of the feet Twenty of these cases are verified histologically The differential diagnosis includes osteomyelitis, gout, and Reiter's disease The roentgenographic features and the possible pathogenetic mechanisms of peripheral metastases are discussed

*Key words* carcinoma peripheral bone metastases
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Although the overall incidence of metastatic involvement of bones in patients with malignant neoplasms is 20–30% (1) metastatic lesions of the small bones of the extremities are extremely rare In spite of their rarity these metastatic processes should be included in the differential diagnosis of lytic lesions of the bones of the hands and feet

## CASE REPORT

The patient was a 63 year-old woman who contacted a physician in July 1978 because of a small lump beneath the right breast The lump was removed and it proved to be a metastasis from an adenocarcinoma At the same time the patient began to experience pain in her right great toe Roentgenographic examination revealed a lytic destruction in the proximal phalanx (Fig 1) On examination at the hospital she was found to have a rectal carcinoma

No metastases were detected in the abdominal cavity at operation but later on new subcutaneous metastases appeared and the patient died in Nov 1978 despite cytostatic therapy Autopsy showed metastases in the mesenterium pleurae pericardium subcutis and adrenal glands A biopsy of the proximal phalanx of the right great toe showed metastatic adenocarcinoma

## DISCUSSION

Gall et al (4) collected from the literature 17 cases fo carcinoma with metastases to the small bones of the feet They added seven own cases We have found five additional cases from the literature (2 3 5 10 11) 20 of them are verified histologically

It seems that metastases to the small bones of the hands are even rarer (8) The commonest tumors that metastasize to the small bones of the feet originate from the genitourinary tract (4) Carcinoma of the lung is the commonest tumor that metastasizes to the distal bones of the upper extremities (8 11)

The differential diagnosis is often difficult and includes osteomyelitis gout and Reiter s disease (1 2 3 4 9) Osteomyelitis causes the greatest problems in differential diagnosis because it simulates metastatic bone disease both clinically and roentgenologically However there are some differential roentgenographic features that are fairly typical of metastatic disease (8) Peripheral bone metastases are purely osteolytic and the reactive condensation of the bone which may occur in osteomyelitis has not been observed Periosteal reaction commonly seen in inflammatory processes does not occur with these metastatic lesions The trabecular destruction produced by the metastasis is usually well defined and the surrounding trabeculae are relatively normal in appearance In osteomyelitis there is usually demineralization and smudging of fine trabecular detail in the reaction areas adjacent to zones of complete bone destruction Peripheral metastases do not involve or cross the joint and even with extensive destruction a thin margin of subchondral cortical bone usually remains If the expanding mass of intramedullary neoplasm has broken through the thinned-out shell of remaining cortical bone the adjacent soft tissue involvement is usually homogeneous localized and well defined In inflammatory processes diffuse




*Fig 1* A lytic destruction is seen in the proximal phalanx of the great toe

welling reticulation and obliteration of the subcutaneous fat line are the characteristic soft tissue changes The ballooned-out effect on the remaining cortical shell produced by the expanding neoplastic mass is not seen in osteomyelitis

Two cases of peripheral metastases simulating gout have been reported (2 11) Roentgenographic features and a negative aspiration for urate crystals lead to the correct diagnosis Metastases to the small bones of the extremities may also closely simulate early rheumatoid arthritis (5 6) Reiter s disease can produce osteolytic lesions in metatarsal bones (9) The rapid healing of the lesion and other features of Reiter s disease suggest the correct diagnosis

The reason for the relative rarity of metastases to the small bones of the extremities is not clear One explanation may lie in an inadequate examination of the distal skeleton when searching for bone metastases (7)

Mulvey (8) has suggested that there are two different patterns of hematogenous spread of metastases the more common involving communications with the vertebral venous plexus through which tumor emboli may be deposited in portions of the axial skeleton but not in the peripheral bones Departure from this usual pattern occurs when venous erosion by a pulmonary malignant neoplasm allows tumor emboli to be carried to the left side of the heart thereby reaching the systemic circulation This would explain the fact that pulmonary carcinoma is the commonest tumor that metastasizes to the fingers (8 11) It is also believed that local hemodynamic factors might be involved in the pathogenesis of distal metastases (4 8)

This case report should remind clinicians that pain and swelling in a toe simulating osteomyelitis or gout may occasionally be due to a metastasis from a carcinoma

## REFERENCES


1 Abrams H L Skeletal metastases in carcinoma Radiology 55 534 1950
2 Bevan D A Ehrlich G E & Gupta V P Metastatic carcinoma simulating gout JAMA 237 2746 1977
3 Brown T I S & Ritchie G L Cervical presentation of rectal carcinoma Br Med J 2 832 1978
4 Gall R J Sim F H & Pritchard D J Metastatic tumours to the bones of the foot Cancer 37 1492 1976
5 Jacox R F & Tristan T A Carcinoma of the breast metastatic to the bones of the foot A case report *Arthritis Rheum 3 170 1960*
6 Karten I & Bartfeld H Bronchogenic carcinoma simulating early rheumatoid arthritis Metastases to the fingers JAMA 179 170 1962
7 Krishnamurthy G T Tubis M Hiss J & Blahd W H Distribution pattern of metastatic bone disease A need for total body skeletal image JAMA 237 2504 1977
8 Mulvey R B Peripheral bone metastases Am J Roentgenol Radium Ther Nucl Med 91 155 1964
9 Primer on the rheumatic diseases JAMA (Suppl) 224 661 1973
10 Sworn M J Buchanan R & Moynihan F J Rectal carcinoma presenting as massive metastatic involvement of foot bones Br Med J 2 98 1978
11 Vaezy A & Budson D C Phalangeal metastases from bronchogenic carcinoma JAMA 239 226 1978

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# Epilepsy and Myopathy in a Patient with Rothmund–Thomson s Syndrome

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ABSTRACT A report of a 21 year old female who has had Rothmund–Thomson s syndrome since early childhood is given In addition to the original disease with skin and tendon manifestations, she has also developed myopathy and epilepsy It is discussed whether these two recent manifestations are part of her original syndrome or of different pathogenesis

*Key words* skin les ons myopathy ep lepsy

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More than 100 years ago the German ophthalmologist Rothmund (7) described a familial juvenile skin disease associated with cataracts Several reports on similar skin diseases were published thereafter (1 2 3 4 9) Thomson (10) described in 1936 two cases with atrophic and shiny skin with a teleangiectasic network He also reported that his patients had scanty hair on scalp eyebrows and lashes Both Rothmund s and Thomson s patients were born with normal skin appearances In a review Rook et al (6) summarized the characteristic features of the Rothmund Thomson s syndrome also known as poikiloderma congenita as follows The first skin lesions appear during the first year of l fe They most often involve the face and consist of swelling or d ffuse erythema mimicking a sunburn Later les ons consisting of wide teleangiectasia spread to different sites of the body surface The pat ents are light sensitive and also have anhydrosis (8) Cataracts occur in about 40% of reported cases (3) Often these patients have small short fingers and toes with skeletal deformities and muscular contractures (5) The disease is thought to be inherited in an autosomal recessive way

So far neither generalized muscular disorders nor convulsions have been described in association with the Rothmund–Thomson s syndrome

## CASE REPORT

A female aged 21 was admitted to the Department Medic ne in Nov 1978 after hav ng suffe ed a grand  al seizure Her perinatal h story was un  ntful and  e wa qu te normal at b rth S nce her f rst y ar of  fe she h d had sk n changes on the cheeks u ua ly w th dry th n  n and later skin atrophy and teleangiecta es on arm and legs Her scalp ha r had always been  ery th n and he wore a w g She had no sweat glands and could not  ate heat The diagnos s of po k oderma con  n  or Rothmund–Thomson s syndrome thus wa  h  nl  p able No hered tary sk n d seases are k  wn n the f  she has two healthy s bl ngs

During ch ldhood the pat ent had d  elo  d  tures in the Ach lles tendons w  h  qu  u  mities Three achillotenotom es have been  e  r  ter the latest in 1973 she complained of m  ula ness n both arms These symptoms then g re  l  e progressed slowly An electromyograph  n 1974 h  a clear s gns of myopathy w th f bri at ons and p sharp waves Muscular enzymes were ra sed an  n b opsy revealed myopathy of unspec f  type A  h  h came more disabled and the poss b  of myo t  not be excluded treatment w th large d  es of cort  r was introduced n Sept 19 8

The patient has graduated from the econom s cla  n h gh school She lives with her parents and de p te h disablement works as off ce clerk at the local a rport She s able to walk short d stances but has great diff cult es with steps She cannot raise her arms above the horizontal plane

*Present h story* The se zure which led to ho p tal za tion was a grand mal attack accompan ed by sal va  n and tongue lacerat on wh ch lasted for 3–4 m n A post c tal phase occurred afterwards Neurolog cal exam nat on revealed weak reflexes and a right pos tive Bab nski s gn Otherwise her phys cal status was normal apart from the s gns of myopathy and ectodermal d sease known earl er (F g 1) She was on prednisone medicat on 100 mg every second day and had tolerated it well Her blood pressure and S-electrolytes were normal the S-Ca however was within the lower l m t of the normal range It was revealed that she had had one se zure a few months earl er while visiting a warm country and had related th s attack to her sens tivity to heat The f rst attack occurred before in tia tion of cort sone therapy



Fig 1 The face of the patient showing typical skin changes She s wearing a wig

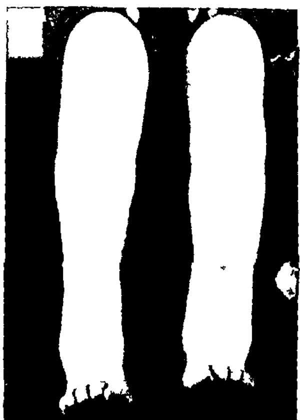


Fig 2 The legs showing lymphoedema and skin changes

EEG showed diffuse slowed background activity without any local focuses The EEG changes were slight but considered pathological by an experienced observer Skull X ray was normal Because of the known myopathy heart examination was carried out ECG showed normal sinus rhythm with a frequency of 98 beat /min phonocardiography showed a split second heart sound and a systolic apical murmur but was thought to be within the normal range Myocardial scintigraphy with ⁹⁹Tcm pyrophosphate showed a diffuse uptake of pyrophosphate suggesting a possible myocardial involvement

*Treatment* As cortisone treatment was unsuccessful in improving the myopathy it was decided to slowly reduce her dose She also received antiepileptic treatment with carbamazepine (Tegretol®) At follow up examination some month after admission the patient felt well and had had no seizures She tolerated her medicine well and the serum level of carbamazepine concentration was within the therapeutic range

## DISCUSSION

Despite the absence of cataract and evidence of a genetic aetiology the condition the diagnosis of Rothmund–Thomson syndrome in this case seems clear The patient had several different ectodermal manifestations (Fig 1) with symptoms from the hair skin and sweat glands and also a lymph oedema (Fig 2) She also had severe contractures of the Achilles tendons which are described as typical symptoms of the Rothmund–Thomson's syndrome (6 11)

For many years this patient has also had a myopathy (Fig 3) verified by EMG and muscular biopsy Myopathy has not been previously described as a feature of the Rothmund Thomson s syndrome but could possibly fit into the picture together with tendon changes Her diffuse positive myocardial pyrophosphate scintigram may indicate an involvement of the cardiac muscle

Epilepsy or other changes in the nervous system are not described in connection with the Rothmund–Thomson s syndrome In this patient it could be a disease of quite different origin Normal pregnancy and birth exclude obstetrical reasons for her epileptic manifestations It is possible that the first seizure was provoked by heat which she was



*Fig 3* Wrists and hands are swollen and the patient is unable to stretch her fingers because of the myopathy

known to tolerate badly and the second by cortisone therapy

We find it interesting for two reasons to report this case Firstly the patient has a rare but rather well known syndrome primarily affecting ectodermal structures but also tendons Perhaps her myopathy and the central nervous manifestations are other features of this syndrome This may be important to know When examin r ot e s t nts with this disease and if similar feat r t the whole spectrum of clinical f t Rothmund–Thomson s syndrome w better understood

Secondly it is important for th l realize that a patient with some k nd severe disease known from childhoo d develop quite new and different sympt could be part of the syndrome with f t r tion or a totally new disease

## REFERENCES


1 Braun W & Vager C Zur Frag t n n Thomson Syndromes D rmat l W n 1184 1965
2 Cheesbrough M Po k lode ma c n ta C se re port Br J Dermatol (Suppl 16 66 13 8
3 Denver A Rothmund Thomson n dr me A oculocutaneous d sorder Am J D Ch d ll 1966
4 Diem E Rothmund Thom o Syndrom scher Be trag Hautarzt 6 4 5 197
5 Oates R Lemus M & Wla e Rothmund–Thomson synd me unusual syndrome Aust Pe iat J
6 Rook A Davis R & Stevan c f congenitale Rothmund Thomson s Derm Venereol 34 392 1959
7 Rothmund A Uber Katarakt in V r u einer eigentumlichen Haut Degenerat o A ct o thalmol 14 159 1868
8 Sexton G Thomson s syndrome Can d A e J 70 659 1954
9 Taylor W B Rothmund s synd ome—Thomson s syndrome Arch Dermatol 75 236 19 7
10 Thomson M Po kiloderma congen ale Br J Dermatol 48 221 19 6
11 Vankos J & Kapu E Das Thomson Syndrom Dermatol Wochenschr 2 574 1961

such solid grounds today Public health actions should therefore await the results of controlled trials

However salt reduction as a dietary treatment is another thing Salt reduction can be recommended as an adjunct to drug treatment of hypertensive patients and might also be tried in subjects with high risk of developing definite hypertension

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## REFERENCES


1 Berglund G Aurell M & Wilhelmsen L Renal function in normo- and hypertensive 50-year-old males Acta Med Scand 199 25 1976
2 Berglund G Wikstrand J Wallentin I & Wilhelmsen L Sodium excretion and sympathetic activity in relation to severity of hypertensive disease Lancet 1 324 1976
3 Berglund G Wilhelmsen L Sannerstedt R Hansson L Andersson O Sivertsson R Wedel H & Wikstrand J Coronary heart disease after treatment of hypertension Lancet 1 1 1978
4 Birkenhager W H Control mechanisms in essential hypertension p 51 Elsevier Amsterdam 1976
5 Burns-Cox C J & Maclean J D Splenomegaly and blood pressure in an Orang Asli community in West Malaysia Am Heart J 80 718 1970
6 Cruz-Coke R Etcheverry R & Nagel R Influence of migration on blood pressure of Easter Islanders Lancet 1 697 1964
Dawbeer T R Kannel W B Kagan A Donabedian R K McNamara P M & Pearson G Environmental factors in hypertension In The epidemiology of hypertension (ed J Stamler R Stamler & T N Pullman) pp 255–268 Grune & Stratton New York 1967
8 Freis E D Salt volume and the prevention of hypertension Circulation 53 589 1976
9 Grim C E Weinberger M H Henry D P Luft F C & Fineberg N S Biochemical correlates of the increase in blood pressure with age Clin Sci Mol Med (Suppl) 4 377 1978
10 Hatano S Hypertension in Japan a review In Epidemiology and control of hypertension (ed O Paul) pp 63–99 Symposia Specialists Miami 1974
11 Kaminer B & Lutz W P W Blood pressure in Bushmen of the Kalahari Desert Circulation 22 289 1960
12 Kean B H The blood pressure of the Cuna Indians Am J Trop Med Hyg 24 341 1944
13 Kesteloot H Joosens J V Lee C S Park B C & Brems-Heyns E A comparative study of blood pressure and sodium intake in Belgium and in Korea Acta Cardiol 23 82 1978
14 Lau K Cooper R McKeever J McKeever P Byington R Stamler R & Stamler J How many measurements of 24-hour urine Na are needed to characterize an individual for the assessment of the relationship between salt intake and blood pressure within a population? Abstract no 921 VII World Congress of Cardiology Tokyo Japan 1978
15 Lowenstein F W Blood pressure in relation to age and sex in the tropics and subtropics A review of the literature and an investigation in two tribes of Brazil Indians Lancet 1 389 1961
16 Maddocks I Blood pressures in Melanesians Med J Aust 1 1123 1967
17 Miall W E Follow up study of arterial pressure in the population of a Welsh mining valley Br Med J 2 1204 1959
18 Morgan T Adam W Gillies A Wilson M Morgan G & Carney S Hypertension treated by salt restriction Lancet 1 227 1978
19 Oliver W J Cohen E L & Neel J V Blood pressure sodium intake and sodium related hormones in the Yanomamo Indians a no-salt culture Circulation 52 146 1975
20 Page L B Damon A & Moellering R C Jr Antecedents of cardiovascular disease in six Solomon Islands societies Circulation 49 1132 1974
21 Page L B Vandervert L Nader K Lubin N Dowell J & Page J Blood pressure diet and body form in traditional nomads of the Qash qai tribe southern Iran Acta Cardiol 23 102 1978
22 Parijs J Joossens J V van der Linden L & Amery A P K C Moderate sodium restriction and diuretics in the treatment of hypertension Am Heart J 85 22 1973
23 Pickering G Discussion remark at the round table discussion on salt at the 6th Scientific Meeting of the International Society of Hypertension in Goteborg June 1979 Clin Sci To be published
24 Prior A M Evans J G Harvey H P B Davidson F & Lindsey M Sodium intake and blood pressure in two Polynesian populations N Engl J Med 279 515 1968
25 Scotch N A preliminary report on the relation of sociocultural factors to hypertension among the Zulu Ann NY Acad Sci 84 1000 1960
26 Shaper A G Cardiovascular disease in the tropics III Blood pressure and hypertension Br Med J 3 805 1972
27 Simpson F O Waal-Manning H J Bolli P & Phelan E L Relationships of blood pressure to sodium excretion in a population survey Clin Sci Mol Med (Suppl) 4 373 1978
28 Stricker E M Thirst sodium appetite and complementary physiological contributions to the regulation of intervascular fluid volume In The neuropsychology of thirst (ed A N Epstein H R Kissileff and E Stellar) p 83 Winston & Sons Washington DC 1973
29 Waern U Findings at a health survey of 60-year-old men and recorded diseases during their preceding 10 years of life Thesis Uppsala 1977
30 Wikstrand J Berglund G Wilhelmsen L & Wallentin I Orthogonal electrocardiogram apex cardiogram and atrial sound in normotensive and hypertensive 50-year-old men Br Heart J 38 779 1976

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# Hypertension, Levels of Serum Gamma Glutamyl Transpeptidase and Degree of Blood Pressure Control in Middle-Aged Males

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ABSTRACT Among the first screened 2439 males born in 1926 and 1927, aged 48–49 years at the time of screening and representing 76% of these age cohorts, uncontrolled or partly controlled hypertension was found in 7 5% Of these individuals 30% preferred to remain with their physicians, regardless of the degree of control they had achieved Among those who were referred to the Hypertension Unit (5.2% of the screened population), elevated S-GT levels (≥1 10 μkat/l) were found in 38 3%, against 18 5% in the two cohorts During 24 months of treatment and follow up only two men among the entire group of hypertensives referred dropped out, both were heavy drinkers (>80 g alcohol daily) The mean BP after treatment was significantly lower among men with normal than high S-GT values or in those who admitted to heavy drinking Of the 99 males treated for more than two years 82 (83%) were responders (supine DBP ≤95 mmHg) Of the non-responders, 70% were either heavy drinkers or had abnormal S-GT values The possible role of alcohol in the pathogenesis of essential hypertension in middle-aged males is discussed

*Key words* essential hypertension primary screening middle aged males gamma glutamyl transpeptidase alcohol consumption BP control

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Recent studies by Ramsay (13) and Beevers (1) indicate an increased rate of liver dysfunction among their hypertensive patients These authors discuss the possible role of an overconsumption of alcohol Klatsky et al (5) have recently shown a clear relation between blood pressure (BP) and the daily number of drinks In the former studies serum levels of aspartate aminotransferase (S-ASAT) alanine aminotransferase (S-ALAT) alkaline phosphatase (S ALP) and bilirubin were measured in both hypertensives and controls and significant differences were shown in S-ALAT S-ALP and bilirubin but not in S-ASAT values

In the present study we have used the serum level of gamma glutamyl transpeptidase (S GT) (11) as a possible indicator of the individual consumption of alcohol both at the time of screening and especially during treatment The initial S-GT value at screening has been compared with a long term result (>24 months) in BP control

In other studies under preparation within this group we use the markedly raised S-GT levels (>1 50 μkat/l) in serum in attempts to trace latent alcoholism in more than 10000 subjects of different age groups and both sexes (7)

## SUBJECTS AND METHODS

Since 1974 we have invited all middle aged men in Malmo to a comprehensive screening examination and so far more than 10000 (75%) have accepted this invitation The screening has included physical features such as height, weight skinfold BP (0+10 min both supine and erect) and heart rate (HR) Metabolic examinations have comprised blood sugar glucose tolerance test including insulin assays liver enzymes including S-GT serum lipids uric acid as well as routine tests (ESR Hb leucocytes electrolytes and urine tests for albumin glucose and blood) All were examined in the fasting state in the morning

Of the first 2439 examined males aged 48 and 49 years at screening 7.5% were hypertensives (defined as systolic BP (SBP) above 160 mmHg and/or diastolic BP (DBP) above 105 mmHg supine or erect after 0 and 10 min

*Abbreviations* BP = blood pressure DBP = diastolic BP SBP = systolic BP S-GT = serum gamma glutamyl transpeptidase ASAT = aspartate aminotransferase ALAT = alanine aminotransferase ALP = alkaline phosphatase HR = heart rate

*Fig 1* Results of two ye... antihypertensive treatme...

at two visits) All these men were invited to the Hypertension Unit and 128 (5 2%) of those screened responded The other 2% with known hypertension preferred to stay with their own physicans

The present study is concerned especially with the 99 hypertensive males who have been followed for 24 months or more This follow up time was considered long enough to establish adherence quality of BP control and any changes in the S-GT levels Twenty nine patients were observed for not more than 1–1 9 years in the Hypertension Unit because they had borderline pressures and were not referred from the Institute of Preventive Medicine until they definitely went above the cut-off levels

Results from a 2 year follow up of S-GT during treatment in 154 males belonging to the five cohorts 19_6–30 are also presented At the Hypertension Unit BP and pulse rates were measured by nurses after 10 min rest and 1–2 min standing DBP was measured at the dissapperance of Korotkoff's sound 5 Mecury manometers were always used (Trimline PyMaH) A routine clinical interview and clinical examination were carried out by the physician though with special attention to obtaining a close history of alcohol beverages diet and smoking habits. The patients were controlled usually every fortnight or month by nurses and less frequently by physicians until normotension (≤160/95) or a significant pressure drop (>10% diastolic) had been achieved Thereafter they were controlled at longer intervals Serum lipids S-GT and weight were controlled every six monts and yearly routine metabolic tests as Hb ESR electrolytes and urine were performed

The treatment started with a $\beta$-blocker (propranolol atenolol or pindolol) Vasodilators (hydralazine or prazosine) and/or diuretics (bendroflumethiazide mefruside or spironolactone) were the next choice if combination therapy was necessary Besides the pharmacological treatment the program involved information concerning other risk factors such as overweight smoking high sugar and fat and excessive salt intake There results will be published later together with data from other age groups of males born in 1928–32 and 1938 as well as of females born in 1928–30 and 1938

*Controls*

The non hypertensive male population in the same age groups served as controls for S-GT levels and the interrelation of BP and S-GT

Table 1 *Significant differences in SBP DBP and HR between the three groups after at least two years antihypertensive treatment*

| | *t* | *p*< |
|---|---|---|
| Group I against group II | | |
| *Supine DBP* | 2 069 | 0 05 |
| Drop in supine SBP | 2 520 | 0 01 |
| Group I against group III | | |
| Supine DBP | 3 500 | 0 001 |
| Drop in supine DBP | 3 868 | 0 001 |
| Supine HR | 1 900 | 0 05 |
| Drop in supine SBP | 3 920 | 0 001 |

Table II S GT and DBP responses after two years antihypertensive treatment together with pretreatment S GT and HR values (mean ± S D ) in groups II and III

| ¹ | Group II (S-GT ≥1 10 µkat/l n=40) | | Group III (S-GT≥1 10 µkat/l n 9* S-GT<1 10 µkat/l n=4) | |
|---|---|---|---|---|
| | S-GT<1 10 (n=25) | S-GT≥1 10 (n=15) | S-GT decreased (n=7) | S-GT increased (n 6) |
| S GT response after two years antihypertensive treatment | 0 86±0 16 ↑ ** | 2 51±1 17 | 1 46±0 86 | 2 23±1 85 |
| Initial S-GT (µkat/l) | 1 70±1 06 | 2 51±1 94 | 3 11±2 80 | 1 28±1 12 |
| | | → * | | |
| Initial HR (beats/min) | 78±12 | 89±13 | 89±12 | 85±16 |
| No of non responders (supine DBP >95 mmHg) | 2 | 7 | 3 | 3 |

¹ Heavy drinkers with elevated S-GT are also included in Group II
*$p<0\ 01$ * $p<0\ 001$

## RESULTS

### *Results of Antihypertensive Treatment*

*Group I (S GT <1 10 µkat/l) and no history of heavy alcohol consumption*

Of 54 patients in this group with normal S GT levels only four (7 3%) were non responders (Fig 1) Three of these four patients had completely normal home BPs and thus only one patient was considered to be a non responder at the Hypertension Unit The BP achieved during treatment in this group is not far from the mean BP in the whole cohort (Fig 1) HR was significantly higher in this group (77±15) than in the total cohort with normal S-GT (69±7) ($p<0\ 01$)

The heavy drinking males some of them also with normal S-GT levels are considered separately (group III)

*Group II (S GT >1 10 µkat/l)*

Among these 40 patients studied for two years or more the number of non responders is somewhat larger than in group I ($n=9$ 22 5%) There was a significant difference in the SBP during treatment compared with group I ($p<0\ 05$) (Table I)

The initially elevated S GT levels returned to normal in 25 (62 5%) of these 40 subjects Only two of the latter patients were BP non responders compared with seven of the 15 subjects who still [ex]hibited elevated S-GT levels after 24 months [Ta]ble II)

In interviews concerning alcohol consumption it was clear from the beginning that the mean consumption in this group with abnormal S GT was high All but four of these patients both prospectively and retrospectively gave either a record of daily alcohol consumption or heavy weekly consumption more than 80% admitting a weekly consumption of 200 g alcohol or more No teetotallers were found

*Group III (heavy drinkers)*

All 15 patients who initially admitted to problems with alcohol and had a mean daily intake of 80 g alcohol or more are classified and handled as heavy drinkers Ten had abnormal S-GT levels and 9 were registered at the Department of Alcohol Diseases These men were observed somewhat more intensively and their S-GT levels were controlled more regularly Seven of them also came to the alcohol intervention unit of the Institute of Preventive Medicine Two patients from group III dropped out during the first year even though both had severe hypertension (WHO III)—one with heart insufficiency grade I and the other with an intermittent claudication Of the remaining heavy drinkers six were non responders as regards BP (Table II) Of these 13 remaining heavy drinkers seven (4 responders 3 non responders) displayed a decrease in their S-GT values Of the six patients who showed either unchanged or still more elevated S-GT levels three were non responders

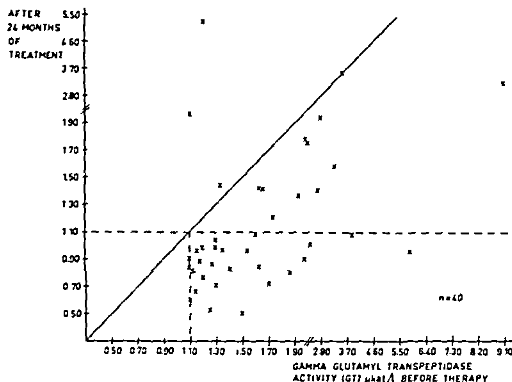



*Fig 2* S-GT levels before and after two years treatment for hypertension in the 40 males born 1926–27 who had elevated initial values

The initial HR was high in this group (87±14) as in the S-GT non responders (85±16) (Table II) The relation between changes in S GT values and alcohol intake could not be taken seriously because all claimed that they had diminished their intake The three heavy drinkers whose S-GT became normal from high initial values (5 85 3 84 and 2 20µkat/l) have all had normal S-GT levels as well as normalized BPs for more than a year (Fig 2) All three reported a dramatic decrease in their alcohol consumption

### *The change in S GT levels*

The men who admitted to a moderate/heavy consumption of alcohol were instructed to abstain for several weekdays and also if possible diminish their intake on their leisure days All patients gave a positive verbal response to this advice As Fig 2 shows 90% of the patients with elevated initial S-GT levels have displayed a substantial decrease and as many as 62 5% achieved normal values The four individuals whose S-GT levels were either higher or unchanged all admitted to an abnormal long lasting alcohol consumption at the time of the last two controls Two of these patients were later referred to the Department of Alcohol Diseases and one succeeded in attaining a normal S-GT level

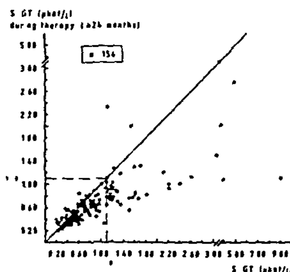



*Fig 3* Long term results regarding S-GT values in 154 males born in 1926–30 Note that even initial normal levels of S-GT tend to fall.

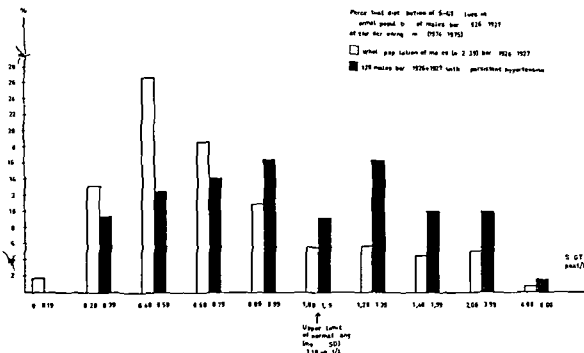


*Fig 4* The hypertensive population has a distinct shift towards high S-GT levels

In the five age cohorts of males born in 1926–30 154 out of 253 have had their S-GT levels controlled This group shows the same trends of falling S-GT levels as the former group (Fig 3)

*Relation between hypertension and S GT levels at screening*

Fig 4 clearly shows that the hypertensive population ($n=128$) has a shift to the right in its percentage distribution of S-GT levels in relation to the 2439 individuals in the screened cohort The mean S-GT value in the hypertensive males was 1 13± 1 08 μkat/l compared with 0 86±0 93 μkat/l in the normal population and this difference was highly significant ($t=9720$ $p<0\ 0001$) Among hypertensive males 38% had abnormal S-GT levels compared to 18 5% among the whole population The incidence of hypertension among males with S-GT levels below the median (S-GT <0 54 μkat/l) is low 4% compared with 11% among males with elevated S-GT levels (>1 10 μkat/l) Fig 5 shows the percentage distribution of BPs in 3018 randomly selected males from the age cohorts 1926–30 at the first screening visit split into two groups (S GT <0 54 and >1 44 μkat/l respectively) The incidence of supine DBP of ≥105 mmHg after 10 min rest is almost six times higher among males with abnormal than low S-GT

## DISCUSSION

Treatment of hypertensive populations screened from primary preventive trials has steadily increased internationally as well as in Sweden (2 4)

The long term results differ depending on factors such as age sex drop-out rates geographical racial and local circumstances and the definition of hypertension The type of first drug used i e β-blockers or diuretics may differ However these two main types of drug have almost the same BP lowering effects and other non pharmaceutical factors may be very important Treatment of hypertension by lowering body weight (14) or salt intake (10) may be successful—especially together with active pharmacological treatment

In the present report we have focused on the possible role of alcohol as both a primary and a secondary mover A recent study from Finland (9) on

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# Hazards of Therapy for Excessive Hypertension in Acute Stroke

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ABSTRACT Six cases with acute onset of neurological symptoms and extremely high blood pressure (BP) are reviewed Hypertensive crisis or stroke were the main differential diagnoses According to what is advocated for both situations, prompt antihypertensive therapy was instituted Although recommended doses of hydralazine, reserpine or furosemide were given, the systolic BPs fell to less than 100 mmHg Intracerebral hemorrhage or infarction was subsequently established in all patients and only one survived Convincing evidence for a beneficial effect of BP reduction in acute stroke is lacking Our data indicate excessive response to therapy in some patients Also, moderate lowering of BP might reduce cerebral blood flow in these patients, often chronically hypertensive and with raised intracranial pressure Extreme caution with antihypertensive therapy seems therefore warranted if the diagnosis of hypertensive crisis is not certain and a stroke is suspected

*Key words* cerebrovascular disorders hydralazine hypertension drug therapy hypertension malignant reserpine

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Antihypertensive treatment is generally agreed to be an essential measure in the primary prevention of cerebrovascular disease (19 20) In secondary prevention the importance of blood pressure (BP) control has been stressed by some authors (1 13) although not by others (8)

As regards the acute phase of a stroke the authorities recommend that hypertension should be treated especially in patients with intracerebral hemorrhages (2 4 5 9 16) When the BP elevation is excessive the situation should be considered as an emergency and urgent therapy is advocated (4 11 15)

Six cases with acute onset of neurological deficit and extremely high BP are reviewed Prompt antihypertensive therapy was instituted and threatening BP falls were registered Against this background lowering of the BP in the acute phase of a stroke is discussed

## CASE REPORTS

Some clinical data on the six cases reviewed below are given in Table I

*Case 1*

This 70 year-old man had been previously healthy During a walk he suddenly got a slight headache and felt dizzy Returning home a little later he lay down After a few minutes his wife noted that his breathing became irregular and she could no longer get into contact with him He was admitted as an emergency 1 hour and 15 min after onset of symptoms

He was then deeply comatose with irregular respiration and peripheral cyanosis The pulse rate was regular 70/min BP high and ECG normal His pupils were small and a papillary edema existed on both sides as did an extensor plantar response

The injections given and the patient s BP are shown in Fig 1 He died in respiratory arrest 65 min after arrival

The postmortem revealed a large left cerebellar hemorrhage with extension to the fourth ventricle

*Case 2*

This 67 year-old man had a history of hypertension and myocardial infarction One afternoon he suddenly felt weak and dizzy Soon afterwards he became unconscious and developed generalized fits He arrived at the hospital 2 1/2 hours after the onset of symptoms

He had an extensor plantar response bilaterally a very high BP and atrial fibrillation Diazepam and morphine were given i v and the fits ceased Hydralazine was given as an antihypertensive agent and the BP fell markedly (Fig 2) The patient became pale and started to sweat With an infusion of around 1 000 ml of fluid the patient regained his former state BP increased gradually to stay on a level around 190–210/90–110 mmHg until the patient died three days later

Autopsy results The left hemisphere contained an infarct measuring 4×5×7 cm and in the left part of the [illegible] there was another 2×2×3 cm A recent infarct was f[illegible] also in the myocardium It was not possible to [illegible] whether any of the lesions were more recent [illegible] others

Table I *Some clinical data on six cases treated for excessive hypertension in the acute phase of strok*

| Case no | Sex | Age (y) | BP (mmHg) Initial | Minimum | Drugs given mode of administration and dosage Antihypertensive | Other | Diagnosis |
|---|---|---|---|---|---|---|---|
| 1 | ♂ | 70 | 260/150 | 140/65 | Hydralazine i v 25 mg<br>Furosemide i v 60 mg | – | Cerebellar hemorrhage (autopsy) |
| 2 | ♂ | 67 | 270/130 | 80/50 | Hydralazine i v 12 5 mg | Diazepam i v 5 mg<br>Morphine i v 10 mg | Hemispheric or pontine infarct myocardial infarct (autopsy) |
| 3 | ♂ | 60 | 240/140 | 45/ | Reserpine i m 1 5 mg<br>Furosemide i v 20 mg | – | Hemispheric hemorrhage (autopsy) |
| 4 | ♂ | 63 | 330/220 | 60/ | Reserpine i m 5 mg<br>Furosemide i v 20 mg | Deslanoside i v 0 4 mg<br>Aminophylline i v 230 mg | Cerebellar hemorrhage (autopsy) |
| 5 | ♂ | 46 | 300/150 | 100/50 | Hydralazine 50 mg i v drip | – | Hemispheric hemorrhage (autopsy) |
| 6 | ♂ | 50 | 210/135 | 60/ | Hydralazine i v 12 5 mg<br>Reserpine i m 1 5 mg<br>Furosemide i v 40 mg | – | Hemispheric infarction (CT scan) |

*Case 3*

A 60-year-old man who had previously been treated in another hospital for hypertension and an aterothrombotic brain infarction with right hemisymptoms On that occasion he also had had a venous thrombosis and pulmonary embolism and had since been maintained on anticoagulants and antihypertensive therapy His condition had improved and he managed well at home At breakfast one day the patient suddenly developed a left facial paresis which was confirmed at home by a doctor who also found a BP of 220/140 mmHg (Fig 3)

A few hours later the patient became comatose and was therefore brought to hospital Bloody cerebrospinal fluid was found and the right pupil was larger than the left An

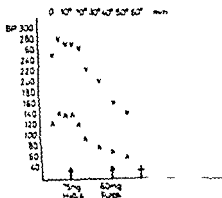



*Fig 1* Case 1 Intravenous injections and BP plotted against time after arrival at the hospital *Hydra* = hydralazine *Furos* = furosemide † = death

i m injection of reserpine was given About one hour la the patient deteriorated during an X ray examination the skull His breathing became irregular and both pup dilated He was moved to the Intensive Care Unit w an i v injection of furosemide was given for pulmona rales The BP gradually sank and the patient died 4 ho and 70 min after arrival

A massive right hemispheric hemorrhage was re at autopsy The blood had extended to the third and fo ventricles Also an old cerebral softening was seen in left hemisphere

*Case 4*

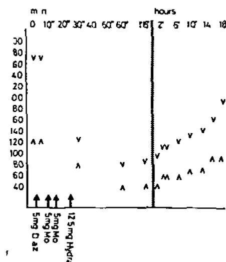
This 63 year-old man with a history of hypertension ha twice been treated for hypertensive crises One day developed severe headache and started to vomit A fe hours later he became unconscious and was brought hospital with an extremely high BP (330/220) Reserpin 2 5 mg i m was immediately administered He also had rapid atrial flutter and pulmonary rales for which he w treated with digitalis aminophylline and furosemide Th BP and the patient s condition were unchanged one h after arrival and another injection of reserpine was ministered BP fell within 2 hours to 60 mmHg systolic

The patient developed shock and died A cerebella hemorrhage with extension to the fourth ventricle wa found at autopsy

*Case 5*

A previously healthy man complained evening of a severe next morning h vomited and was somet

On arrival at the hos and mmHg was fusion wit




*g 2* Case 2 Intravenous injections and BP plotted ainst time after arrival at the hospital *Dia.* = azepam Mo = *morphine* *Hydra* = hydralazine

hydralazine was instituted During the following 4 hours BP dropped gradually to a minimum level of 165/85 mHg at which time the infusion was interrupted The tient remained unconscious and the BP again increased around 260/90 mmHg A new drip with 25 mg hydral ine was started The BP fell to 100/50 mmHg and the tient died 36 hours after arrival

At the postmortem a large right sided hemorrhage in brain was revealed as well as left ventricular hyper phy of the heart

*se 6*

is 50 year-old man had been receiving treatment for pertension for several years One day he suddenly duced dysarthria and a weakness of the right hand so he lled on the hospital

He immediately received antihypertensive treatment d his BP dropped gradually during the next few hours hen it was down at 80 mmHg systolic the patient had teriorated with general malaise and weakness which bsided when the BP rose and stabilized around 150/90 mHg The pareses remained unaltered through these rst phases but improved later during hospitalization The vestigations including lumbar puncture and CT scan owed that the patient had suffered a left hemispheric farction

## DISCUSSION

ll the patients reviewed had fallen ill quite sudden with headache dizziness vomiting and pareses ive of them had already developed coma or emicoma before admission Four had a known his ory of hypertension One had even had hyperten ve crises before All BPs initially recorded in the hospital were extremely high Stroke or hyperten sive crisis were the main differential diagnoses Antihypertensive treatment is considered urgent in both conditions and was therefore instituted promptly

Nevertheless none of the patients improved neurologically when their BPs had been reduced or even while a reasonable level was maintained Then the BPs continued to drop far below what had been aimed at When they had fallen more than 50% in five cases below 100 systolic the patients showed signs of deterioration or shock The pres sure reaction as well as the deterioration might have been the natural terminal course in these patients with severe brain lesions However the condition of patients 2 and 6 improved again when the BP rose This favours the idea that the deterioration was caused by the fall in BP This in turn was linked so closely to the administration of antihypertensive drugs and the expected onset of their action that it seems reasonable to assume that it was a sequela of the therapy This assumption is also supported by the fact that when drugs were withdrawn as in cases 2 5 and 6 a high pressure was restored

May the type of drugs or the doses used explain the excessive BP reductions?

Hydralazine was used most frequently In cases 1 and 5 it was the only medicine administered before the fall in BP occurred It was given in i v doses of 12 5–25 mg Case 5 had i v drips of two bottles 25 mg each The first drip was interrupted after five hours when his BP had fallen to 165/85 mmHg The

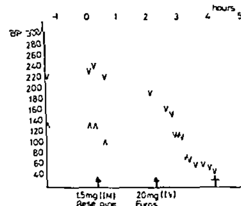



*Fig 3* Case 3 Injections and BP plotted against time after arrival at the hospital *IM* = intramuscularly *IV* = in travenously *Furos* = furosemide † = death

infusion was repeated on the next day and this time BP fell to 100 mmHg systolic after six hours

Hydralazine has been generally recommended as safe and effective under circumstances similar to the present The doses are in accordance with those advised by the manufacturers and other authorities (4 9 11) However exaggerated responses in hypertensive patients have been reported in two German papers (7 12) According to the former paper, lower doses than recommended elsewhere usually no more than 5-10 mg should therefore be given as a single dose

Reserpine was administered in three cases in i m doses of 1 5-2 5 mg In case 3 the only other medication given was 20 mg of furosemide, which could not possibly have caused the drastic fall in BP In case 4 the reserpine injection was repeated within one hour which is considered too short an interval since the effect is delayed On the other hand, a total of 5 mg of reserpine is still within the range of what is recommended as a single dose (4 9)

Furosemide 20-60 mg was administered in four cases Such a marked effect on the BP is noticed very rarely after furosemide However it might have contributed to the effect of the more potent antihypertensive drugs This might also be true for other medications given to two of the present cases

Thus it seems as if these patients responded in an exaggerated way to recommended doses of both hydralazine and reserpine What did this undesired BP reaction mean for their outcome?

Cases 1 and 4 had cerebellar hemorrhages They were deeply comatose already on admission and in very poor condition Even if the right diagnosis had been suspected immediately it is very unlikely that they would have survived diagnostic procedures and operation (18) The sequelae of therapy therefore probably did not mean anything for their outcome Cases 3 and 5 had massive hemispheric hemorrhages which in the former case had extended to the ventricular system It is not plausible that they would have survived regardless of therapy Case 6 managed the drop in his BP without any permanent deterioration of his neurological state which must be regarded as fortunate In case 2 it could not be stated whether any of the ischemic lesions in the brain and heart were more recent than the others It is possible that the myocardial infarction was the primary disease which had given rise to cerebral embolism However a cerebral infarction might also have developed during the BP fall

The question cannot be answered whether the [illegible] tient in that case would have survived without [illegible] complication

It seems that the fall in BP did not mean m[illegible] the outcome in our patients However the drop [illegible] far below the level needed for autoregulation [illegible] cerebral blood flow especially as most of th[illegible] tients were permanently hypertensive (17) [illegible] might therefore have been hastened in some [illegible] tients and the markedly negative effect of the [illegible] was alarming for personnel and relatives In [illegible] instances moreover it might be of great progn[illegible] importance to avoid any reduction of cerebral b[illegible] flow by these means

What then should be done in cases with [illegible] onset of neurological symptoms and exce[illegible] hypertension?

The first difficulty is to distinguish betwe[illegible] hypertensive crisis and a stroke Usually the [illegible] of a hypertensive crisis is less sudden than [illegible] of a stroke General symptoms such as nausea [illegible] headache prevail in the former condition and [illegible] neurological signs are less prominent Fundusc[illegible] examination may show papilledema hemorrh[illegible] and exudates CSF findings are usually nor[illegible] where as—at least in hemorrhagic strokes—CS[illegible] often bloody An acute CT scan might also be [illegible] ful at least in finding intracerebral bleedings Str[illegible] with hypertension are nowadays much more [illegible] mon than hypertensive crises (4 9 11)

In spite of these characteristics it might be im[illegible] sible directly to establish which diagnosis [illegible] hand The only solution then is to start a caut[illegible] antihypertensive treatment Low doses of short[illegible] ing drugs should be preferred Diazoxide [illegible] sodium nitroprusside are now widely recommen[illegible] (11) but were not at hand for the present c[illegible] Intense supervision is mandatory Improve[illegible] should promptly follow BP reduction if the pa[illegible] is suffering a hypertensive crisis Otherwi[illegible] stroke is most likely at hand

*What should then be done to the BP?*

It is known that very high BPs (>200/115) [illegible] associated with a higher case fatality rate at [illegible] early stage of stroke (6) The pressure migh[illegible] harmful by increasing cerebral edema Howeve[illegible] might also be a beneficial reflex el[illegible] come the raised intracerebral [illegible] with a massive lesion

As far as we know [illegible] have been published [illegible]

thorities favour antihypertensive therapy as mentioned in the introduction. There is also one report on the benefit of therapy in a clinical material of ischemic strokes. Around 10% of the patients though had a tendency to orthostatic collapse during treatment with clonidine or methyldopa (5). Other authors, on the basis of their experience, recommend instead that arterial hypertension should be induced in such cases (10). Furthermore, in patients with muscle rigidity there is a risk of overestimating the blood pressure when monitored indirectly. Therapy might then cause damagingly low cerebral perfusion pressures (14).

The lack of convincing evidence for a beneficial effect of BP reduction, theoretical reasons as well as our experience of excessive responses to therapy have made us doubtful about treating hypertension in the acute phase of a stroke. This is further supported by the fact that the BP decreases gradually even without treatment in most patients within the first few days (3, 15). If an active treatment approach is chosen, extreme caution with drugs, doses and supervision is necessary.

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## REFERENCES


1. Carter A B. Hypotensive therapy in stroke survivors. Lancet 1: 485, 1970.
2. Cerebrovascular diseases: Prevention, treatment and rehabilitation. WHO Techn Rep Ser 469: 22, 1971.
3. de Faire U, Britton M, Helmers C & Wester P O. Blood pressure during the acute phases of cerebrovascular disease. Acta Med Scand (Suppl) 621: 27, 1978.
4. Frohlich E D. The hypertensive crisis. Clinician Hypertension, pp 71–75. Searle & Co, Amsterdam 1973.
5. Gottstein U & Seel A W. Antihypertensive therapy in stroke patients. Acta Neurol Scand (Suppl) 64: 174, 1977.
6. Hatano S. Experience from a multicenter stroke register. A preliminary report. Bull WHO 54: 541, 1976.
7. Hennig D. Klinische Erfahrungen mit intravenöser Applikation von Depressan Ampullen. Z Urol 68: 169, 1975.
8. Hypertension–Stroke Cooperative Study Group. Effect of antihypertensive treatment on stroke recurrence. JAMA 229: 409, 1974.
9. Joint Committee for Stroke Facilities. Clinical Management Study Group. Medical and surgical management of stroke. Stroke 4: 273, 1973.
10. Kassell N F, Peerless S J & Drake C G. Reversal of ischemic deficits by induced arterial hypertension. Third Joint Meeting on Stroke and Cerebral Circulation. Stroke 9: 104, 1978.
11. Keith T A III. Hypertension crisis. Recognition and management. JAMA 237: 1570, 1977.
12. Klumpp F, Klaus D, Rossler R & Sadowski P. Reninaktivität im Nierenvenenblut und ihre Stimulierbarkeit durch Hydralazin und Theophyllin. Klin Wochenschr 51: 875, 1973.
13. Marshall J. A trial of long term hypertensive therapy in cerebrovascular disease. Lancet 1: 10, 1964.
14. McGraw C P & Barnes R W. Thalamic hemorrhage and arterial blood pressure monitoring. Third Joint Meeting on Stroke and Cerebral Circulation. Stroke 9: 105, 1978.
15. Oxbury J M. Diseases of the central nervous system. Treatment of stroke. Br Med J 4: 450, 1975.
16. Ross Russel R W. Cerebral arterial disease, pp 167, 216–217. Churchill Livingstone, Edinburgh, London and New York 1976.
17. Strandgaard S. Autoregulation of cerebral blood flow in hypertensive patients. The modifying influence of prolonged antihypertensive treatment on the tolerance to acute, drug induced hypotension. Circulation 53: 720, 1976.
18. Sypert G W. Cerebellar hemorrhage and infarction. Compr Ther 3: 42, 1977.
19. Veterans Administration Cooperative Study Group on Antihypertensive Agents. Effect of treatment on morbidity in hypertension. I. Results in patients with diastolic blood pressures averaging 115 through 129 mmHg. JAMA 202: 1028, 1967.
20. — Effect of treatment on morbidity in hypertension. II. Results in patients with diastolic blood pressures averaging 90 through 114 mmHg. JAMA 213: 1143, 1970.

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# Body Fluid Volumes and the Response of Renin and Aldosterone to Short- and Long-Term Thiazide Therapy of Essential Hypertension

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**ABSTRACT** Plasma volume (PV), extracellular fluid volume (ECV) serum electrolytes, renin and aldosterone were measured before and after 1 week and 4 months of hydrochlorothiazide (HCT) treatment, 50 mg twice daily, in nine male patients with uncomplicated essential hypertension All studies were carried out under strictly standardized conditions in a metabolic ward After 1 week of HCT treatment significant reductions were found in PV and ECV, but after 4 months only ECV was significantly reduced During HCT therapy, renin and aldosterone were permanently elevated whereas serum sodium and potassium were lowered After 1 week, renin was inversely correlated with PV and ECV and directly correlated with heart rate After 4 months, renin was inversely correlated with serum sodium These results indicate a permanent decrease in ECV during long term HCT therapy It is further suggested that the mechanisms responsible for the renin response during short and long term HCT treatment are different, changes in body fluid volumes and increased neural activity being responsible for the initial rise in renin, and serum sodium being the predominant factor during chronic treatment

*Key words* essential hypertension hydrochlorothiazide plasma volume extracellular fluid volume renin aldosterone

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There is abundant evidence that short term thiazide treatment of essential hypertension is accompanied by reductions in plasma volume (PV) (11 12 13 22) and extracellular fluid volume (ECV) (22 32) and by a marked stimulation of the renin–angiotensin–aldosterone system (3 17 33) The situation after *chronic thiazide therapy* however is less certain A permanent reduction in PV (15 20 21) and ECV (20 27) has been reported but could not be confirmed in other studies (9 13 32) Data on the state of the renin–angiotensin aldosterone system during long term thiazide treatment are also controversial (8 17)

It was felt that strict control of the experimental conditions could help to clarify these issues In the present study therefore PV and ECV were measured under circumstances of a standardized sodium and potassium intake both in the acute and chronic phase of thiazide treatment In addition the results of plasma renin and aldosterone estimations were related to body fluid volumes and some relevant clinical and biochemical variables

## PATIENTS

Nine male patients (aged 20–50 years) participated after they had given their informed consent All had a diastolic blood pressure (BP) of more than 100 mmHg on different occasions when untreated A diagnosis of essential hypertension was made after exclusion of secondary causes of elevated arterial pressure by a routine work up Endogenous creatinine clearance was normal in all patients (>100 ml/min) and eye ground changes were of grade I or II ECG signs of left ventricular hypertrophy were present in three patients

### *Study protocol*

After a period of 4 weeks without medication patients were admitted to the metabolic ward where they received a diet containing 50 mmol of sodium and 90 mmol of potassium per day BP and heart rate were measured twice daily On day 9 blood was drawn for determination of renin and aldosterone at 9 a m after an overnight fast and recumbency and at 10 a m after 1 hour of 45 head up tilt In the morning of day 10 PV and bromide space (BS) were

*Abbreviations* BP=blood pressure HCT hydrochlorothiazide PV plasma volume ECV extracellular fluid volume PRC plasma renin concentration PA= plasma aldosterone concentration BS=bromide space

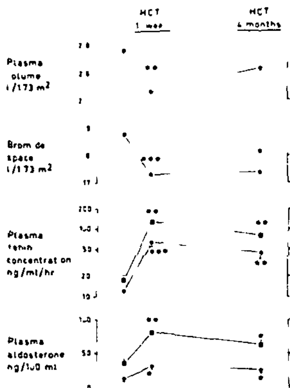



*Fig 1* Plasma volume bromide space renin and aldosterone before treatment and after 1 week and 4 months of hydrochlorothiazide treatment (mean ± S E M ) Significance of differences compared with pretreatment values $p<0.05$ * $p<0.01$ $p<0.001$

...ured in the fasting and recumbent patient Thereafter hydrochlorothiazide (HCT) 50 mg twice daily was administered and determinations of renin aldosterone PV and BS were repeated after 1 week of therapy The patients were then dismissed and seen at biweekly intervals in the Outpatient Clinic After 4 months of HCT monotherapy they were readmitted to the metabolic ward and again placed on a 50 mmol sodium 90 mmol potassium diet while the diuretic was continued On day 9 the aforementioned studies were repeated

## METHODS

PV was measured a the distribution space of radiolabeled human serum albumin Blood was collected into heparinized tubes via an indwelling catheter from an antecubital vein before and exactly 10 20 30 and 40 min after an i v injection of 5 μCi I albumin in the opposite arm Plasma was separated immediately and 2 ml samples of plasma and standard solution were counted for radioactivity in an automatic gamma counter Plasma radioactivity at time zero was calculated from monoexponential regression of radioactivity in the 10–40 min samples

ECV was estimated by measuring the distribution volume of radioactive bromide (3) An i v injection of 20 ml of a dilution of Na ⁸²Br (20–30 μCi) was given in the late afternoon After a 16-hour equilibration period during which all urine voided was collected a blood sample was taken Samples of plasma urine and standard solution were counted for radioactivity and BS was calculated as described by Tarazi et al (27) Immediately after blood collection for estimation of BS radio-labeled human serum albumin was injected for determination of PV Plasma renin concentration (PRC) was determined by a radioimmunoassay (25) after processing the plasma according to Skinner (26) Plasma aldosterone concentration (PA) was determined by a radioimmunoassay as described by Bayard et al (2) Serum sodium and potassium were determined by flame photometry

BP and heart rate were measured after 10 min of supine rest with a sphygmamonometer with standard cuff size Phase IV Korotkoff sounds were taken as diastolic BP BP and heart rate figures used for subsequent analysis were the mean of the values of the last two days of each study period

Statistical analysis was performed by Student s *t* test for paired observations and linear regression Values of $p<0.05$ were regarded significant

## RESULTS

### *Blood pressure*

BP data of this study have been discussed in detai previously (6) Supine BP before admission to th metabolic ward was 156±5/106±4 mmHg (mean S E M ) It fell significantly to 140±5/96±3 mmH in the clinical period without HCT BP values 1 week on HCT (137±6/97±3 mmHg) and after months on HCT (134±4/93±2 mmHg) were significantly lower than before admission but d not differ significantly from values in the clin period without HCT BP changes were unrelated changes in body fluid volumes

### *Plasma volume and bromide space* (Fig 1 Table I)

PV was lowered after 1 week on HCT ($p<0.01$) the reduction after long term therapy was not st tically significant BS was significantly redu both after 1 week ($p<0.001$) and after 4 ($p<0.02$) of HCT therapy

### *Renin and aldosterone* (Fig 1 Table II)

HCT increased supine and tilt PRC signific the most marked rise being found after 1 we therapy A similar pattern was observed for s and tilt PA Supine and tilt PRC correlated both before and during HCT treatment (r

Table I *Plasma volume and bromide space (l/l 73 m²) before treatment and after 1 week and 4 months of hydrochlorothiazide treatment*

ND not done

| Pat no | Before HCT | | After 1 week on HCT | | After 4 mo on HCT | |
|---|---|---|---|---|---|---|
| | PV | BS | PV | BS | PV | BS |
| 1 | 2 5 | 19 9 | 2 2 | 17.5 | 2 0 | 17 9 |
| 2 | 2 7 | 18 4 | 2 4 | 16 1 | 2 2 | 15 7 |
| 3 | 2 5 | 18 8 | 1 9 | 16 6 | 2 8 | 15 6 |
| 4 | 2 8 | 18 9 | ND | 18 0 | 2 7 | ND |
| 5 | 2 3 | 17 4 | 2 3 | 16 0 | 2 5 | 16 4 |
| 6 | 3 1 | 17 6 | 2 8 | 17 0 | 2 8 | 15 4 |
| 7 | 3 1 | 19 8 | 3 0 | 19 4 | 2 9 | 19 8 |
| 8 | 2 7 | 18 6 | 2 6 | 17 7 | 3 0 | 19 5 |
| 9 | 3 2 | 20 2 | 2 6 | 17 7 | 2 9 | 18 6 |
| Mean | 2 8 | 18 8 | 2 5 | 17 3 | 2 6 | 17 4 |
| ± S E M | 1 0 | 0.3 | 1 3 | 0 4 | 1 2 | 0 6 |

0 97 $p<0\ 001$) Supine PRC after 1 week on HCT correlated with pretreatment supine PRC ($r=0\ 67$ $p<0\ 05$) No such correlation was found between *supine PRC after 4 months of HCT treatment and* pretreatment PRC ($r=0\ 48$ n s ) Supine and tilt PA were directly related to supine PRC ($r=0\ 59$ $p<0\ 001$) and tilt PRC ($r=0\ 60$ $p<0\ 001$)

*Heart rate serum sodium and potassium* (Table III)

Heart rate rose during HCT treatment but this rise was significant only after 1 week of treatment ($p<0\ 01$) A significant inverse correlation between heart rate and PV was found after 1 week on HCT ($r=-0\ 76$ $p<0\ 02$) but not after 4 months ($r=-0\ 16$ n s ) Serum sodium and potassium decreased significantly both after short ($p<0\ 001$) and long term ($p<0\ 05$ *and* $p<0\ 001$ *respectively*) *diuretic* treatment

*Renin versus body fluid volumes heart rate and serum sodium* (Fig 2)

Pretreatment supine PRC was inversely correlated with PV ($r=-0\ 87$ $p<0\ 01$) but insignificant corre lations were found with BS heart rate and serum sodium After 1 week s HCT treatment supine PRC was inversely related both to PV ($r=-0\ 90$ $p<0\ 01$) and BS ($r=-0\ 86$ $p<0\ 01$) At that time

*Table II *Plasma renin (ng/ml/h) and plasma aldosterone (ng/100 ml) concentrations before treatment and after 1 week and 4 months of hydrochlorothiazide treatment*

S=supine T=after 1 hour of 45 head up tilt

| Pat no | Before HCT | | | | After 1 week on HCT | | | | After 4 mo on HCT | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PRC | | PA | | PRC | | PA | | PRC | | PA | |
| | S | T | S | T | S | T | S | T | S | T | S | T |
| 1 | 22 9 | 40 3 | 21 8 | 41 7 | 82 0 | 157 0 | 46 0 | 104.0 | 44.0 | 108 3 | 25 0 | 58 0 |
| 2 | 12.5 | 15 6 | 15 0 | 39 0 | 88 3 | 240.3 | 33 0 | 120 0 | 67 1 | 161 2 | 20 0 | 66 0 |
| 3 | 12.0 | 19.5 | 1 9 | 4 8 | 91.5 | 211 0 | 36 0 | 120 0 | 40 9 | 55.5 | 34 0 | 108 0 |
| 4 | 8 4 | 16 4 | 21 0 | 40 0 | 37 2 | 57 8 | 37.5 | 47.5 | 31 5 | 41 0 | 24 5 | 61.5 |
| 5 | 18 0 | 20 7 | 20 0 | 69 0 | 97 8 | 223 6 | 34 0 | 75 0 | 107 0 | 189.0 | 14.8 | 33 0 |
| 6 | 9 0 | 11 2 | 14 0 | 51 0 | 45 4 | 68 4 | 53 0 | 126 0 | 29 8 | 40 7 | 33 0 | 72 0 |
| 7 | 10 4 | 15.5 | 8 2 | 20.8 | 29 9 | 37 0 | 11 6 | 56 1 | 20 3 | 23 8 | 35 0 | 60 0 |
| 8 | 9 0 | 9 4 | 11.6 | 37 0 | 43 4 | 59.8 | 11.5 | 58 4 | 20.6 | 31 9 | 10 0 | 50 0 |
| 9 | 7 5 | 13.8 | 6.7 | 17.5 | 55.8 | 83 6 | 20 0 | 38 0 | 54 4 | 84 5 | 37 0 | 59 0 |
| Mean | 12 2 | 18 0 | 13 4 | 35 6 | 63.5 | 126.5 | 31 4 | 80 6 | 46 2 | 82 3 | 25 9 | 63 7 |
| ± S E M | 1 7 | 3 0 | 2.3 | 6 4 | 8 8 | 27 1 | 4 8 | 12 4 | 9 2 | 19 9 | 3 2 | 6 3 |

Table III *Heart rate (beats/m n) serum s d um (mEq l) and serum p t ss n (mEq l) bef re treatn ent and er 1 eek 4 t s f/ a ×ll e th de tre t e t*

| Pat no | Before HCT | | | After 1 week on HCT | | | After 4 mo on HCT | | |
|---|---|---|---|---|---|---|---|---|---|
| | HR | Na | K | HR | Na | K | HR | Na | K |
| 1 | 81 | 139 | 4 4 | 89 | 133 | 3 1 | 90 | 135 | 3 ? |
| ? | 61 | 13? | 4 8 | ? | 136 | 3 1 | 3 | 134 | 3 4 |
| 3 | 69 | 13? | 4 1 | 85 | 133 | 3 0 | 85 | 135 | 3 3 |
| 4 | 63 | 140 | 4 0 | 68 | 135 | ? 6 | 65 | 137 | ? 7 |
| 5 | 6 | 140 | 4 4 | 84 | 133 | ? 7 | 77 | 134 | ? 7 |
| 6 | 60 | 13? | 4 0 | 0 | 136 | ? ? | 65 | 139 | 3 1 |
| | 3 | 140 | 4 1 | 0 | 135 | 3 1 | 0 | 138 | 3 4 |
| 8 | 5 | 139 | 3 8 | 80 | 140 | ? 5 | 5 | 14? | ? 6 |
| 9 | 6? | 140 | 3 9 | 5 | 137 | ? 9 | 62 | 138 | ? 9 |
| Mean | ( ) | 1 9 | 4 | 77 | 136 | ? 9 | 4 | 137 | 3 0 |
| S E M | 3 | 0 3 | 0 1 | 3 | 0 7 | 0 1 | 3 | 0 9 | 0 1 |

sup ne FRC was d rectly related to heart rate ($r$=0 6 $p$<0 0?) but the correlation w th serum sod um was not s gn f cant ($r$= 0 45) In contrast after 4 months HCT treatment no s gn ficant cor relations were found between sup ne FRC and PV ($r$= 0 53) BS ($r$= 0 54) or heart rate ($r$=0 18) At th s t me however FRC was nversely correlated w th serum sod um ($r$= 0 ?7 $p$ 0 0?)

## DISCUSSION

The f nding of s gn f ant reductions n PV and ECV ng short term thiaz de treatment of essential hype tension confirms the results of many other stud es (1 3 13 ?? 3?) After 4 months of HCT treatment ECV was st ll reduced but PV had in creased values not s gn f antly d fferent from pretreatment values These f nd ngs agree w th several studies but d sagree w th others (see ntroduc tion) It should be noted however that sod um intake was not standardized in many of the earlier stud es and that pat ents who were also rece v ng other antihypertens ve treatment were n luded n some stud es It was emphas zed by Leth (?0) that a reduction in PV was f und in all stud es on the long term effect of thiaz des but that th s reduction was not s gnificant n s me tudies Theref re our f nd ngs provide firm ev den e for a lowered ECV and are compat b e w h a de reased PV during chron c thiazide treatment

In the present study ren n and aldosterone were permanently e evated dur g thiaz de treatment Al though it is generally ac ep ed that short term d uretic therapy is accompan ed by stimulation of the ren n-ang otens n-aldosterone system (8 17 33) the question whether th s also holds true for long term treatment has not been establ shed (8 17) Desp te demonstrat on of the contrary n some earl er stud es (3 13 31) ev dence is now ac



F g ? Changes of plasma renin concentration after 1 week (*left*) and 4 months (*right*) of hydrochlorothiazide treatment

cumulating that at least renin is permanently elevated during diuretic therapy of essential hypertension (1 7 27 29) and more importantly that this can counteract the BP lowering effect of these drugs (16 19 28) Data on aldosterone during long term thiazide treatment are also contradictory In some studies aldosterone was found to be elevated (29 30) whereas no significant differences from pre treatment values were found in some others (3 13) It is quite possible that variations in dietary sodium intake are responsible for the conflicting data since sodium intake can modulate the adrenal response to angiotensin II (6 24) Nevertheless our data suggest that the renin–angiotensin system is the most important single factor for the release of aldosterone during diuretic treatment since aldosterone correlated with renin but not with serum sodium or potassium levels

A close correlation was found between supine renin and the renin level after tilting On the other hand pretreatment renin was a poor predictor of renin values during therapy presumably because other important regulatory mechanisms of renin release are influenced by diuretics (17)

The rise in plasma renin after short term thiazide treatment is usually attributed to body fluid volume depletion (3 28) and there is also evidence that the sympathetic nervous system is involved (18 33 34) This view is supported by our finding that after 1 week of HCT therapy renin was not only inversely related to plasma and extracellular fluid volume but also directly correlated with heart rate The mechanism responsible for the rise in renin during long term treatment is less well defined From the evidence available it appears that volume depletion and neural mechanisms are less predominant here Thus no correlation was found between changes in plasma volume and changes in renin during chronic thiazide treatment (1) Moreover it has been reported that volume re expansion during diuretic treatment results only in partial suppression of renin (14) Finally $\beta$ adrenergic blockade in thiazide treated patients was found to have little influence on plasma renin (4) In keeping with this view are the weak and insignificant correlations between renin body fluid volumes and heart rate that we observed after 4 months of HCT treatment

Hypokalaemia and hyponatraemia stimulate the release of renin (10) and these factors are likely to be of concern during thiazide therapy In the present study we found no relation between renin and serum potassium levels On the other hand a significant inverse correlation between serum sodium and renin emerged after 4 months of HCT treatment This observation is in agreement with earlier studies (5 14) and suggests that during long term diuretic treatment serum sodium is an important determinant of the renin response

## ACKNOWLEDGEMENT

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## REFERENCES


1 Acchiardo S Dustan H P & Tarazi R C Similar effects of hydrochlorothiazide and spironolactone on *plasma renin activity in essential hypertension* Cleve Clin Q 39 153 1972
2 Bayard F Beitins I Z Kowarski A & Migeon C J Measurement of plasma aldosterone by radioimmunoassay J Clin Endocrinol 31 1 1970
3 Bourgoignie J J Catanzaro F J & Perry H M Renin–angiotensin–aldosterone system during chronic thiazide therapy of benign hypertension Circulation 37 27 1968
4 Bravo E L Tarazi R C & Dustan H P $\beta$ Adrenergic blockade in diuretic treated patients with essential hypertension N Engl J Med 292 66 1975
5 Brown J J Davies D L Lever A F & Robertson J I S Plasma renin concentration in human hypertension I Relationship between renin sodium and potassium Br Med J 2 144 1965
6 van Brummelen P Schalekamp M & de Graeff J Influence of sodium intake on hydrochlorothiazide induced changes in blood pressure serum electrolytes renin and aldosterone in essential hypertension Acta Med Scand 204 151 1978
7 van Brummelen P Woerlee M & Schalekamp M A D H Long term versus short term effects of hydrochlorothiazide on renal haemodynamics in essential hypertension Clin Sci 56 463 1979
8 Conway J Antihypertensive effects of diuretics In Antihypertensive agents Handbook of experimental pharmacology vol 39 (ed F Gross) p 477 Springer Verlag Berlin 1977
9 Conway J & Lauwers P Hemodynamic and hypotensive effects of long term therapy with chlorothiazide Circulation 21 21 1960
10 Davis J O & Freeman R H Mechanisms regulating renin release Physiol Rev 56 1 1976
11 Dustan H P Cumming C R Corcoran A C & Page I H A mechanism of chlorothiazide enhanced effectiveness of antihypertensive ganglioplegic drugs Circulation 19 360 1959
12 Frohlich E D Schnaper H W Wilson I M & Freis E D Hemodynamic alterations in hypertensive patients due to chlorothiazide N Engl J Med 262 1261 1960

13 Gifford R W Mateos V R Orvis A L Sones D A & Rosevaer J W Effect of thiazide diuretics on plasma volume body electrolytes and excretion of aldosterone in hypertension Circulation 24 1197 1961

14 Had P M Dustan H P & Tarazi R C Suppression of renin release by intravascular volume expansion during chronic diuretic treatment Cleve Clin Q 41 5 1974

15 Hansen J Hydrochlorothiazide in the treatment of hypertension Acta Med Scand 183 317 1968

16 Ibsen H Leth A Holnagel H Kappelgaard A M Damkjaer Nielsen M & Giese J Renin angiotensin system in mild essential hypertension The functional significance of angiotensin II in untreated and thiazide treated hypertensive patients Clin Sci Mol Med (Suppl) 55 319 1978

17 Johnston C I Effect of antihypertensive drugs on the renin-angiotensin system Drugs 12 274 1976

18 Leonetti G Mayer G Morganti A Terzoli L Zanchetti A Bianchetti G Di Salle L Morselli P L & Chalsey C A Hypotensive and renin suppressing activities of propranolol in hypertensive patients Clin Sci Mol Med 48 431 1975

19 Leonetti G Terzoli L Sala C Bianchini C Sernesi L & Zanchetti A Relationship between the hypotensive and renin-stimulating actions of diuretic therapy in hypertensive patients Clin Sci Mol Med (Suppl) 55 307 1978

20 Leth A Changes in plasma and extracellular fluid volumes in patients with essential hypertension during long term treatment with hydrochlorothiazide Circulation 42 479 1970

21 Lund J hansen P Hemodynamic changes in long term diuretic therapy of essential hypertension Acta Med Scand 187 509 1970

22 M Queen E G & Morrison R B I The hypotensive action of diuretic agents Lancet I 1209 1960

23 Nicholson J P & Zilva J F Estimation of extracellular fluid volume using radiobromine Clin Sci 19 [illegible] 1960

24 Oelkers W Brown J J Fraser R Lever A F Morton J J & Robertson J I S Sensitization of the adrenal cortex to angiotensin II in sodium deplete man Circ Res 34 [illegible] 1974

25 Schalekamp M A D H Schalekamp-Kuyken M P A de Moor Fruytier M Meiningen T Vaandrager Kranenburg D J & Birkenhager W H Interrelationships between blood pressure renin renin substrate and blood volume in terminal renal failure Clin Sci Mol Med 45 417 1973

26 Skinner S L Improved assay methods for renin concentration and activity in human plasma Circ Res 20 391 1967

27 Tarazi R C Dustan H P & Frohlich E D Long term thiazide therapy in essential hypertension Circulation 41 709 1970

28 Vaughan E D Carey R M Peach M J Ackerly J A & Ayers C R The renin response to diuretic therapy Circ Res 42 376 1978

29 Vaughan E D Laragh J H Gavras I Buhler F R Gavras H Brunner H R & Baer L Volume factor in low and normal renin essential hypertension treatment with either spironolactone or chlorthalidone Am J Cardiol 32 523 1973 ✓

30 Weber M A Drayer J I M Rev A & Laragh J H Disparate patterns of aldosterone response during diuretic treatment of hypertension Ann Intern Med 87 558 1977

31 Werning C Baumann K Schönbeck M Gysling L Weidmann P & Siegenthaler W Die Wirkung langer dauernder Hydrochlorothiazid-Gaben auf die Plasma Renin-Aktivitat und die Aldosteron Exkretionsrate bei Normalpersonen Klin Wochenschr 47 318 1969

32 Wilson I M & Freis E D Relationship between plasma and extracellular fluid volume depletion and the antihypertensive effect of chlorothiazide Circulation 20 1028 1959

33 Zanchetti A Leonetti G Morganti A Terzoli L Schwarz L Manfrin M & Bernasconi M Longitudinal study of plasma renin activity in hypertensive patients under antihypertensive treatment including diuretics In Systemic effects of antihypertensive agents (ed M P Sambhi) p 251 Stratton New York 1976

34 Zanchetti A S Neural regulation of renin release Circulation 56 691 1977

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# Serum Myoglobin Compared with Creatine Kinase in Patients with Acute Myocardial Infarction

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ABSTRACT In a retrospective study of 307 patients with suspected acute myocardial infarction (AMI) the diagnostic value of S-myoglobin quantitation was compared with S creatine kinase (CK, EC 2 7 3 2) The results were compared with the final diagnoses, which were made according to the WHO criteria A reference group of healthy blood donors and children hospitalized for removal of adenoids was investigated Children (2–16 years) had significantly lower myoglobin levels (25±10 μg/l) than adults (19–65 years, 41±17 μg/l) In the children a positive correlation was found between age and S-myoglobin concentration but not in adults Women (34±17 μg/l) had lower S-myoglobin concentration than men (47±15 μg/l) The difference in sensitivity for detection of AMI by S-myoglobin or S-CK analyses is not significant when the results from blood samples drawn on admission and on the following three mornings are compared, but myoglobin can be detected earlier in serum than CK after an AMI If only the results from blood samples drawn on admission are compared, the S-myoglobin analysis had a significantly higher sensitivity than the CK analysis The S-myoglobin analysis had a lower specificity than the CK analysis, and i m injections were found to be an important reason for false positive results S-myoglobin may be of value in the very early verification of AMI, but the frequent blood sampling and the low specificity are problematic The simultaneous quantitation of S-myoglobin and the heart specific CK isoenzyme fraction (MB) seems to be a good combination

*Key words* acute myocardial infarction myoglobin serum

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The diagnosis of acute myocardial infarction (AMI) is mainly based upon a typical h[illegible] G findings and serum enzyme analyses [illegible]tory tests for AMI usually include S aspartate amino transferase (ASAT EC 2 6 1 1) S-lactate dehydrogenase (LD EC 1 1 2 3) and S-creatine kinase (CK EC 2 7 3 2) and perhaps isoenzyme fractionation of CK and LD The latter two tests seem to be superior with regard to specificity but are rather difficult to carry out in the daily clinical routine Recently myoglobin has been shown to be elevated in serum and urine after myocardial infarction (6 7 13 15 17) and quantitation of myoglobin in serum may even be useful in the very early diagnosis of AMI (15 17) In some other diseases such as muscular dystrophy (1 11) and inflammatory myopathies (5) increased levels of S myoglobin are found too The factors influencing myoglobin concentration in serum are manifold and not yet completely known but both renal and extrarenal factors are of importance (4 16 19)

By means of radioimmunoassay myoglobin can be detected in serum even in normal healthy individuals (14 16 18) Recently we have developed rapid and sensitive radioimmunoassays for S myoglobin (12) which can be carried out fast enough within one hour to be of significance in the early diagnosis of AMI

The purpose of the present study is to compare the value of S myoglobin and S-CK activity in the early diagnosis of AMI

## STUDY POPULATION

Blood samples were drawn by venous puncture after minimal stasis Shortly after clotting the samples were

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*Abbreviat*[illegible] =acute m[illegible]l infarction CK= [illegible] ne [illegible] pa[illegible] LD [illegible] ive positive

Table IV *Calculated nosographic and diagnostic values of myoglobin compared with CK*

Sensitivity = patients with definite AMI and positive test / all with definite AMI

PV pos = patients with true positive test / all with positive test

Specificity = patients with AMI and negative test / all with no AMI

PV neg = patients with true negative test / all with negative test

| | Myoglobin | | CK | |
|---|---|---|---|---|
| | Days 0 1 2 3 | Day 0 | Days 0 1 2 3 | Day 0 |
| Sensitivity | 0.993 | 0.900 | 0.966 | 0.625 |
| Specificity | 0.679 | 0.722 | 0.814 | 0.90. |
| PV pos | 0.666 | 0.679 | 0.773 | 0.806 |
| PV neg | 0.984 | 0.917 | 0.974 | 0.766 |

the department that administered the analgesic intramuscularly we found 45% false positive results of the myoglobin analysis compared with only 20% in those from the department where the routine administration was intravenous. This difference is significant ($p<0.001$, $\chi^2$ test). The CK analysis showed similar results with 29 and 9% false positive values respectively. This was the only difference between the two hospital series. Of the 34 patients with false positive CK results, 4 had activities which did not change during the observation period.

In the control group we found a positive correlation between S-myoglobin concentration and age in the age group 2–16 years (Table V). Above this age the S-myoglobin level was independent of age. We therefore used the group of blood donors as a reference group for the patients with suspected myocardial infarction. Adults had higher S-myoglobin levels than children, and among the blood donors males had higher levels than females ($p<0.001$, Student's t test). No difference in S-myoglobin concentration could be found between males and females in the age group below 16 years.

All patients who had been treated with i.m. injections of penicillin showed increased levels of myoglobin in serum. The concentration was increased a few hours after the injection and reached a maximum of 2–3 times the upper normal level during the first 24 hours. All patients who had undergone operation with muscular damage also showed elevated levels of S-myoglobin. None of the patients who had exercised on a bicycle ergometer had increased S-myoglobin levels or signs of myocardial ischaemia on their ECG curves before or after exercise.

## DISCUSSION

In agreement with other authors (15–17) we found S-myoglobin to be elevated in patients with verified myocardial infarction compared with patients in whom the diagnosis of myocardial infarction was suspected but not verified. The patients with no verified infarction had higher S-myoglobin levels than the healthy blood donors. This may be due to the fact that i.m. injections give increased S-myoglobin levels. Many of our patients had been treated with i.m. injections of analgesics before admission, but the information about this treatment was very poor. Furthermore, one of the two departments usually administers analgesics intramuscularly. This may be one explanation for the high PV pos. of

Table V *Serum myoglobin concentration in control subjects*

| | No. of subj | Myoglobin (µg/l) | | Age (yr) | |
|---|---|---|---|---|---|
| | | Mean ± 1 S.D. | Range | Mean | Range |
| Adults | 94 | 41 ± 17 | 1.–92 | 37 | 19–65 |
| Females | 43 | 34 ± 17 | 12–76 | 37 | 21–63 |
| Males | 41 | 47 ± 15 | 19–9. | 38 | 19–65 |
| Children | 78 | . ± 10 | 6–59 | 8 | 2–16 |

the myoglobin analysis but also other factors are known to induce increased S-myoglobin levels, for instance muscular diseases (1, 5, 11) and decreased glomerular filtration (4). The difference in mean age between our patients and controls can be an explanation too, but we found no correlation between S-myoglobin concentration and age in the control group. Also the majority of the false positive CK results came from the department which gives i.m. injections. In the diagnostic group of definite AMI the S-myoglobin analysis gave a significantly higher PV-neg. than the CK analysis when the results from the blood samples taken on admission were examined, whereas no difference could be found in the results from the samples on days 0, 1, 2, 3. This indicates that S-myoglobin analysis is superior to the S-CK analysis in the very early diagnosis of AMI. It appears from the typical S-myoglobin profile (Fig. 1) that S-myoglobin is the first parameter which can be detected in serum, compared with CK and the CK-MB fraction. S-myoglobin is significantly elevated 2-3 hours after onset of the acute episode, reaches its maximum level within 12 hours and normalizes after 36-48 hours. This can explain the false negative results of the S-myoglobin analysis, because the first two blood samples could have been drawn at an interval of up to 24 hours, so small elevations of short duration may have [illegible] In a comparison of CK and LD isoenzymes, Kraft et al. (8) found the diagnostic reliability [illegible] be greater with isoenzyme determinations, [illegible] negative and positive results, but then also [illegible] it necessary to draw blood samples at short [illegible]. They had doubts about the therapeutic [illegible], and perhaps a combination of S-[illegible] isoenzyme quantitations would be useful in clinical practice. Rapid radio-[illegible]ays and immunoinhibition methods for [illegible] MB quantitation have recently been described. [illegible] oglobin and S-CK MB estimations may therefore both be carried out as AMI tests.

In conclusion, determination of S-myoglobin may be helpful in the early diagnosis of AMI, but with some restrictions. To reduce the number of false positive results, i.m. injection should be avoided and attention should also be paid to other factors known to influence S-myoglobin levels. The test should only be used if the onset of infarction occurs less than about 12 hours before admission, and to reduce the false negative results, blood must be sampled at short intervals within the first 24 hours after infarction. In this way one also obtains the peak value. Furthermore, quantitation of S-myoglobin and S-CK MB should be carried out simultaneously in order to determine and compare the diagnostic value of these tests in the very early diagnosis of AMI.

## ACKNOWLEDGEMENT

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## REFERENCES


1. Adachi, T., Shimizu, T., Kato, T., Ohsawa, M. & Fukuyama, Y.: Myoglobinemia in children with progressive muscular dystrophy. Clin Chim Acta 85: 17, 1978.
2. Antonini, E. & Brunori, M.: Hemoglobin and myoglobin and their reaction with ligands. In: Frontiers of biology, vol. 21 (ed. A. Neuberger & E. Tatum), p. 19. North Holland Publishing Co., London and Amsterdam 1971.
3. Bolton, A. E. & Hunter, W. H.: The labelling of proteins to high specific radioactivities by conjugation to a 125I-containing acylating agent. Biochem J 1[illegible] 529, 1973.
4. Hallgren, R., Karlsson, F. A., Roxin, L. E. & Venge, P.: Myoglobin turnover—influence of renal and extrarenal factors. J Lab Clin Med 91: 2[illegible]6, 1978.
5. Kagen, L. J.: Myoglobinemia in inflammatory myopathies. JAMA 237: 1448, 1977.
6. Kagen, L. J.: Myoglobin. Biochemical, physiological and clinical aspects. Columbia University Press, New York and London 1973.
7. Kessler, H. A., Lurtson, P. R., Mantelheimer, H. & Adams, E. C.: Acute myocardial infarction diagnosed by myoglobinemia. Arch Intern Med 135: 1161, 1975.
8. Kraft, J., Aasrup, H. & Schrader, P.: Diagnostic value for acute myocardial infarction of creatine kinase and lactate dehydrogenase isoenzymes compared with total enzymes. Acta Med S[illegible] 167, 1978.
9. Kunter, H. & Rabbenich [illegible] determination on the GSA [illegible] 9-61, 1973.
10. Mercer, D. W.: Separation [illegible] creatine kinase isoenzymes by [illegible] chromatography. Clin Chem 20: [illegible]
11. Nørregaard-Hansen, K., [illegible]gaard-Pedersen, B.: Potential [illegible] assay of myoglobin in amnio[illegible] prenatal diagnosis of Duchenne [illegible] Lancet 2: 1250, 1978.
12. Nørregaard-Hansen, K. & Nørgaa[illegible] Rapid and sensitive radioimmuno[illegible] myoglobin. Scand J Clin Lab Invest.
13. Pedersen, F., Bahnsen, M., [illegible] & Rosendorf[illegible]: The diagnostic value [illegible]

determination in the urine of patients with acute myocardial infarction. Upsala Larger [illegible] 556 1976.

14 Rosano T. G. & Kenny M. A. A radioimmunoassay for human serum myoglobin: Method development and normal values. Clin Chem 23 [illegible] 1977.

15 Rosano T. G., Sanders L. A., [illegible] E. S., Kenny M. A., Clayson K. J. & [illegible] E. F. Myoglobin concentrations and muscle enzyme activities in serum after myocardial [illegible] and cardiac [illegible]. Clin Chem 23 [illegible] 1977.

16 [illegible] E. [illegible] P. [illegible] G. & Hallgren R. Radioimmunoassay of myoglobin in serum and urine. Scand J Clin Lab Invest 37 [illegible] 1977.

17 Stone M. J., Waterman M. R., Harimoto D., Murray G., Willson W., Platt M. R., Blomqvist G. & Willerson J. T. Serum myoglobin level as diagnostic test in patients with acute myocardial infarction. Br Heart J 39 [illegible] 1977.

18 Stone M. J., Willerson J. T., Gomez-Sanchez [illegible] & Waterman M. R. Radioimmunoassay of myoglobin in human serum. Results in patients with acute myocardial infarction. J Clin Invest 56 1334 [illegible]

19 Sylven C. The kinetics of myoglobin in [illegible] volunteers and in patients with acute myocardial infarction. Scand J Clin Lab Invest 38 [illegible] 1978.

20 WHO Regional Office for Europe. Report of the [illegible] Working Group on Ischaemic Heart Disease Registers. Copenhagen 1971.

21 Yamazaki I., Yokota K. N. & Shikama K. Preparation of crystalline oxymyoglobin from horse heart. J Biol Chem 239 [illegible] 4151 1964.

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# Skin Cholesterol and DNA in Young Patients with Myocardial Infarction

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ABSTRACT Disturbances in cholesterol metabolism are connected with an increased risk of clinical complications to atherosclerosis Serum cholesterol has been used as an index of such disturbances However, recently the significance of local tissue and cellular factors in cholesterol metabolism and atherogenesis have been better appreciated As easily accessible sources of cells and tissues, skin biopsies have been suggested to increase the possibilities to assess the extent of atherosclerosis in an individual In order to test this hypothesis, skin biopsies were taken from 24 male patients, who had sustained a myocardial infarction before the age of 40, and from 42 healthy, randomly selected male volunteers, matched for age and serum cholesterol Cholesterol and DNA contents were measured in epidermis and dermis separately no significant differences were found between the groups A significant, positive correlation between serum and dermis cholesterol was found in both groups Our data do not support the hypothesis that skin biopsies discriminate individuals with atherosclerosis better than serum cholesterol It is possible that previous data suggesting a correlation between skin cholesterol and atherosclerosis, might reflect the well known correlation between serum cholesterol and atherosclerosis

*Key words* atherosclerosis cholesterol lipid metabolism *myocardial infarction, risk factors skin*
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The etiology of atherosclerosis is generally held to be multifactorial (7) Epidemiological studies suggest a number of factors which may be significant (14 21) Thus high serum cholesterol is coupled with an increased risk of diseases secondary th atherosclerosis The mechanisms behind this observation are not well understood On the one hand it has been hypothesized that increased serum cholesterol levels might cause an increased influx of plasma cholesterol into the arterial wall This would explain the fact that the development of atherosclerotic lesions invariably leads to an increase in cholesterol content of the arterial wall (17) On the other hand this hypothesis does not explain why complications to atherosclerosis may develop even at low serum cholesterol levels (15) Furthermore the correlation between plasma and tissue cholesterol is low in normal arterial tissue (3) Therefore it is possible that raised serum cholesterol might be a secondary effect of general disturbances in tissue lipid metabolism Such disturbances have recently been demonstrated in familial hypercholesterolemia (4) and could be an expression of more generally acting factors like age and heredity Signs of these factors may in that case also be found in tissues other than the arterial wall Therefore additional information about the state of the arterial wall might be reached by studying biopsies from other tissues In such a case biochemical analysis of easily available skin biopsies would be advantageous This was in fact pointed out in 1974 by Boissou et al (1) who studied skin lipids and aortic atheromatosis at autopsy They proposed that the extimation of skin lipids could be a diagnostic test of the degree of development of atherosclerosis in an individual" Later Girardet et al (13) made the same suggestion based on a study on patients undergoing surgery for atherosclerotic coronary disease

The aim of the present study was to test this hypothesis on patients who had suffered a myocardial infarction at a comparatively low age The controls were matched for age and for two of the most important risk factors for atherosclerosis i e smoking habits and serum cholesterol

## POPULATION AND METHODS

A specific technique was developed in order to obtain forearm skin biopsies in a gentle manner A piece of skin

Table I. *Background variables (mean ± S.D.)*
No. of subjects given in parentheses

| | Patients | Controls | Non-smokers | Smokers |
|---|---|---|---|---|
| Age at the time of biopsy (y.) | 37.2±4.1 (21) | 36.1±3.0 (42) | 37.0±3.1 (34) | 36.3±3.0 (3[illegible]) |
| Total serum cholesterol (mmol/l) | 6.57±1.34 (24) | 6.87±0.81 (42) | 6.66±1.06 (34) | 6.87±1.02 (32) |
| Serum HDL-cholesterol (mmol/l) | 1.29±0.20 (23) | 1.45±0.27 (42) | 1.39±0.[illegible] (34) | 1.39±0.31 (31) |
| Serum apo-A (g/l) | 2.07±0.25 (21)* | 2.53±0.36 (4[illegible])* | 2.35±0.37 (32) | 2.40±0.42 (31) |
| Serum triglycerides (mmol/l) | 2.41±0.67 (24) | 2.07±0.92 (42) | 2.19±0.54 (34) | 2.20±0.99 (32) |
| Fasting glucose (mmol/l) | 4.36±1.15 (21) | 4.17±0.74 (42) | 4.26±0.56 (33) | 4.21±0.44 (30) |
| Sum glucose (mmol/l) | 29.0±6.2 (21) | 26.7±7.3 (4[illegible]) | 27.7±6.6 (33) | 27.3±7.4 (30) |
| Fasting insulin (mU/l) | 12.7±7.9 (21) | 9.6±4.0 (42) | 9.[illegible]±4.[illegible] (33) | 11.4±6.5 (30) |
| Sum insulin (mU/l) | 277±101 (21) | 236±76 (4[illegible]) | 235±79 (33) | 244±95 (30) |

* Significant difference ($2p < 0.05$)

about 4 mm in diameter was snatched from a lifted skin fold with a tonsil punch (Hartmann). No local anesthesia was required. All subjects had been informed about possible complications beforehand, but no such were reported by the 72 subjects. The investigation was approved by the local Ethical Committee.

Skin biopsies were taken from male patients who had suffered a myocardial infarction before the age of 40. The diagnosis was based upon a typical clinical picture including history, ECG, serum enzyme determinations and left ventriculogram. The patients were selected in 1976 from a register consisting of all persons aged 65 and below with myocardial infarction in Göteborg (10). The interval between infarction and skin biopsy varied (median 8 months, range 4 months–6 years). The *age at the time of infarction* was originally applied when selecting matched controls, which meant that the infarction patients were older than the controls at the time of biopsy. An alternative approach would be to match for the age *at the time of biopsy*. When the 20 patients originally planned to be included in the study had been collected, we therefore decided to add another 11 patients to get a better age match *at the time of biopsy*. We also decided to exclude the two oldest patients in the original group before the final calculations were made. Three others were excluded because they did not meet the angiographic criteria. The experimental group in the further discussions consisted of 24 patients. The age distribution is apparently asymmetric, which is explained by the age criteria used.

Smoking habits were assessed at the time of biopsy. This was considered to be most relevant in view of the fast cell turnover of the skin. The patients were assigned to five subgroups with regard to their smoking habits. Group 1 (n=1[illegible]): non-smokers; group 2 (n=12): previous smokers (abstained for at least four months); groups 3 (n=4), 4 (n=[illegible]) and 5 (n=[illegible]): smokers with a consumption of 1–14, 15–24 and >25 g tobacco a day, respectively. Background variables were determined including serum cholesterol (11), α-lipoprotein cholesterol (5, 19), apo-A-I (20) and triglycerides (5). Blood glucose was determined by a glucose oxidase method using the Glox® assay kit (Kabi, Stockholm, Sweden). Plasma insulin was determined by radio-immunoassay using a primary antibody solid phase technique (Phadebas® assay kit, Pharmacia, Uppsala, Sweden). An oral glucose tolerance test with insulin determinations was performed. Blood samples were taken via an indwelling polyethylene catheter before, 30, 60, 90 and 120 min after ingestion of 100 g glucose in 200 ml water.

Serum cholesterol levels were normally distributed (mean 6.57 mmol/l, S.D. 1.34) (Table I). This value is higher than in a Swedish normal population, as might be expected in a group selected for early myocardial infarction. The serum levels of α-lipoprotein cholesterol, apo-A and triglycerides as well as the calculated parameters of glucose metabolism are shown in Table I.

The control group consisted of 42 males matched for serum cholesterol and age at the time of biopsy. They were selected according to their serum cholesterol level among a random population of 30–40-year-old healthy males from the Section for Preventive Cardiology. All had undergone physical examination 4–6 months before the biopsies. All controls had been informed about the procedures beforehand and had given their full consent to participation.

The age distribution of the controls was not normal due to the selection principles used. The distribution of the controls between the five subgroups as regards smoking habits was 8, 13, 5, 11 and 5, respectively. The serum cholesterol levels of the controls were normally distributed and are shown together with other background variables in Table I.

The skin biopsies were stored for 5 h max. in a small volume of Ringer glucose at 4°C until further treatment. Immediately after thawing, epidermis and dermis were separated by heating the specimens in Ringer glucose at 55°C for 30 sec, followed by careful dissection under a microscope (1). In this way, occasional sebaceous glands and hairs could be removed as well. The result of separation level was checked by light microscopy (Fig. 1).



*Fig 1* Epidermis (top) and dermis (bottom) after separation in Ringer glucose at 55°C for 30 sec. Htx van Gieson ×

Epidermis and dermis specimens were treated separately in the further analysis. Lipids were extracted in chloroform methanol 2 1 and the eluates were washed according to Folch et al (11) Total cholesterol was measured on the eluate according to Bondjers and Björkerud (2) Dry weight was determined on a Cahn RG 4050 automatic microbalance After homogenization DNA was measured according to Kissane and Robins (16)

*Dry weight recovery of the procedure was determined* to be 99 5±12% and cholesterol recovery 93±6% Dissected and non-dissected materials were compared to calculate the recovery values Different skin specimens were matched for wet weight before separate analysis This involved certain problems of standardization which may to a great extent explain the rather large standard deviation of the estimated recovery values The plain mean value given above probably unc terol recovery as the distribution ment (Fig 2) The coeffici individual determinations of c was determined to be 4% for mis The corresponding figu weight were 17% and 14% difference between double termined to be 3 1% for c

Mean values and stan and the significance of evaluated with Student cients were determined correlations with age coefficient was used age distribution cau

*Table I. Background data (mean ± S.D.)*
No. of subjects given in parentheses

| | Patients | Controls | Non-smokers | Smokers |
|---|---|---|---|---|
| Age at the time of biopsy (y) | 3?.2 ± 4.1 ([illegible]) | 36.1±3.0(4?) | 37.0±3.1(34) | 36.9±3.0(32) |
| Total serum cholesterol (mmol/l) | 6.57±1.34([illegible]) | 6.87±0.81(4?) | 6.66±1.06(34) | 6.87±1.02(3?) |
| Serum HDL cholesterol (mmol/l) | 1.?? [illegible] (2?)ᵃ | 1.45±0.27(4?) | 1.39±0.30(34) | 1.32±0.31(31) |
| Serum apo A (g/l) | [illegible] 0.2? (2?)ᵃ | 2.33±0.36(4?)ᵃ | 2.35±0.37(3?) | 2.40±0.42(31) |
| Serum triglycerides (mmol/l) | 2.41±0.87(?) | 2.07±0.92(42) | 2.19±0.94(34) | 2.20±0.??(32) |
| Fasting glucose (mmol/l) | 4.36±1.15(?) | 4.17±0.74(42) | 4.?6±0.86(33) | 4.21±0.94(30) |
| Sum glucose (mmol/l) | 29.0±6.?(?) | ?6.7±7.3(4?) | 27.7±6.6(33) | 2?.3±7.4(30) |
| Fasting insulin (mU/l) | 1?.? [illegible] | 9.6±4.0(4?) | 9.9±4.?(33) | 11.4±6.5(30) |
| Sum insulin (mU/l) | ?? ±101(?) | 236±76(4?) | ?55±79(33) | ?44±95(30) |

ᵃ Significant difference ($p < 0.05$)

about 4 mm in diameter was snatched from a lifted skin fold with a [illegible] punch (Hartmann). No local anesthesia was required. All subjects had been informed about possible [illegible] beforehand, but no such were reported by the [illegible] subjects. The investigation was approved by the [illegible] Ethical Committee.

Skin biopsies were taken from male patients who had suffered a myocardial infarction before the age of 40. The diagnosis was based upon a typical clinical picture including history, ECG, serum enzyme determinations and left [illegible]. The patients were selected in 1976 from a register [illegible] of all persons aged 65 and below with myocardial infarction in Göteborg (10). The interval between infarction and skin biopsy varied (median 8 months, range 4 months–6 years). The age *at the time of infarction* was originally applied when selecting matched controls, which meant that the infarction patients were older than the controls at the time of biopsy. An alternative approach would be to match for the age *at the time of biopsy*. When the 70 patients originally planned to be included in the study had been selected, we therefore decided to add another 11 patients to get a better age match *at the time of biopsy*. We also decided to exclude the [illegible] oldest patients in the original group before the final calculations were made. Three [illegible] were excluded because they did not meet [illegible] criteria. The experimental group in [illegible] consisted of [illegible] patients. The age distribution is apparently [illegible], which is explained by the [illegible] used.

Smoking habits were assessed at the time of biopsy. This was considered to be most relevant in view of the fast cell turnover of the skin. The patients were divided into five subgroups with regard to their smoking habits. Group I [illegible] non-smokers [illegible] previous smokers [illegible] [illegible] and [illegible] [illegible] Background variables [illegible] [illegible] [illegible] [illegible] and [illegible] [illegible] blood glucose was determined by a

glucose oxidase method using the Glox® assay kit (Kabi, Stockholm, Sweden). Plasma insulin was determined by radioimmunoassay using a primary antibody [illegible] technique (Phadebas® assay kit, Pharmacia, Uppsala, Sweden). An oral glucose tolerance test with insulin determinations was performed. Blood samples were taken via an indwelling polyethylene catheter before, 30, 60, [illegible] and 120 min after ingestion of 100 g glucose in 200 [illegible] water.

Serum cholesterol levels were normally distributed (mean 6.57 mmol/l, S.D. 1.34) (Table I). This value [illegible] higher than in a Swedish normal population, as might be expected in a group selected for early myocardial infarction. The serum levels of [illegible] lipoprotein cholesterol, apo [illegible] and triglycerides as well as the calculated parameters of glucose metabolism are shown in Table I.

The control group consisted of 42 males matched for *serum cholesterol and age at the time of biopsy*. They were selected according to their serum cholesterol levels among a random population of 30–40-year-old healthy males from the Section for Preventive Cardiology. All had undergone physical examination 4–6 months before the biopsies. All controls had been informed about the procedures beforehand and had given their full consent to participation.

The age distribution of the controls was not normal due to the selection principles used. The distribution of the *controls between the five subgroups as regards smoking habits* was 8, 13, 5, 11 and 5, respectively. The serum cholesterol levels of the controls were normally distributed and are shown together with other background variables in Table I.

The skin biopsies were stored for [illegible] [illegible] volume of Ringer glucose at [illegible] °C until further treatment. Immediately after thawing, epidermis and dermis were separated by heating the specimens in Ringer glucose at 55 °C for 30 sec, followed by careful [illegible] [illegible] [illegible] [illegible] and hairs could be removed as well. The remaining [illegible] [illegible] was [illegible] by light microscopy (Fig. 1).



*Fig 1* Epidermis (top) and dermis (bottom) after separation in Ringer glucose at 55 C for 30 sec Htx van Gieson ×250

Epidermis and dermis specimens were treated separately in the further analysis Lipids were extracted in chloroform methanol 2 1 and the eluates were washed according to Folch et al (11) Total cholesterol was measured on the eluate according to Bondjers and Bjorkerud (2) Dry weight was determined on a Cahn RG 4050 automatic microbalance After homogenization DNA was measured according to Kissane and Robins (16)

Dry weight recovery of the procedure was determined to be 99 5+12% and cholesterol recovery 93+6% Dissected and non-dissected materials were compared to calculate the recovery values Different skin specimens were matched for wet weight before separate analysis This involved certain problems of standardization which may to a great extent explain the rather large standard deviation of the estimated recovery values The plain mean value given above probably underestimates the cholesterol recovery as the distribution was skew in the experiment (Fig 2) The coefficient of variance of intraindividual determinations of cholesterol per dry weight was determined to be 4% for epidermis and 8% for dermis The corresponding figures regarding DNA per dry weight were 17% and 14% respectively The percentage difference between double sample determinations was determined to be 3 1% for cholesterol and 7 3% for DNA

Mean values and standard deviations were calculated and the significance of differences between means was evaluated with Student's *t* test Linear correlation coefficients were determined between the variables except for correlations with age where Spearman's rank correlation coefficient was used This was because of the non normal age distribution caused by the selection princip

Table I. *Background variables (mean ± S.D.)*

No. of subjects given in parentheses

| | Patients | Controls | Non-smokers |
|---|---|---|---|
| Age at the time of biopsy (y) | 37.2±4.1 ([illegible]4) | 36.1±3.0 (4[illegible]) | 37.0±3.1 (34) |
| Total serum cholesterol (mmol/l) | 6.57±1.34 (24) | 6.87±0.81 (42) | 6.66±1.06 (34) |
| Serum α cholesterol (mmol/l) | 1.[illegible]9±0.20 (23)* | 1.45±0.27 (42)* | 1.39±0.20 (34) |
| Serum apo-A (g/l) | 2.0[illegible]±0.25 (21)* | 2.[illegible]3±0.36 (4[illegible])* | 2.35±0.37 (32) |
| Serum triglycerides (mmol/l) | 2.41±0.87 ([illegible]4) | 2.07±0.92 (4[illegible]) | 2.19±0.84 (34) |
| Fasting glucose (mmol/l) | 4.36±1.15 (21) | 4.17±0.74 (4[illegible]) | 4.[illegible]6±0.86 (33) |
| Sum glucose (mmol/l) | 29.0±6.2 (21) | [illegible]6.7±[illegible].3 (42) | 27.7±6.6 (33) |
| Fasting insulin (mU/l) | 12.7±7.9 (21) | 9.6±4.0 (42) | [illegible]9±4.[illegible] (33) |
| Sum insulin (mU/l) | 2[illegible]7±101 (21) | 236±[illegible]6 (42) | 235±79 (33) |

* Significant difference (2p < 0.05)

about 4 mm in diameter, was snatched from a lifted skin fold with a tonsil punch (Hartmann). No local anesthesia was required. All subjects had been informed about possible complications beforehand, but no such were reported by the 72 subjects. The investigation was approved by the local Ethical Committee.

Skin biopsies were taken from male patients, who had suffered a myocardial infarction before the age of 40. The diagnosis was based upon a typical clinical picture including history, ECG, serum enzyme determinations and left ventriculogram. The patients were collected in 1976 from a register consisting of all persons aged 65 and below with myocardial infarction in Göteborg (10). The interval between infarction and skin biopsy varied (median 8 months, range 4 months–6 years). The age at the time of infarction was originally applied when selecting matched controls, which meant that the infarction patients were older than the controls at the time of biopsy. An alternative approach would be to match for the age at the time of biopsy. When the 70 patients originally planned to be included in the study had been collected, we therefore decided to add another 11 patients to get a better age match at the time of biopsy. We also decided to exclude the two oldest patients in the original group before the final calculations were made. Three others were excluded because they did not meet the angiographic criteria. The experimental group in the further discussions consisted of [illegible]4 patients. The age distribution is apparently asymmetric, which is explained by the age criteria used.

Smoking habits were assessed at the time of biopsy. This was considered to be most relevant in view of the fast cell turnover of the skin. The patients were divided into five subgroups with regard to their smoking habits. Group 1 (n=1) non-smokers, group 2 (n=[illegible]) previous smokers (abstinent for at least four months), groups 3 (n=4), 4 (n=3) and 5 (n=4) smokers with a consumption of 1–14, 15–24 and >25 g tobacco a day, respectively. Background variables were determined including serum cholesterol (8), α-lipoprotein cholesterol (13), apo-A (9, 20) and triglycerides (6). Blood glucose was determined by a

glucose oxidase method, using the Glu[illegible] Stockholm, Sweden). Plasma insulin [illegible] (radioimmunoassay), using a primary a[illegible] technique (Phadebas® assay kit, P[illegible] Sweden). An oral glucose tolerance t[illegible] terminations was performed. Blood [illegible] via an indwelling polyethylene cathet[illegible] and 120 min after ingestion of 100 g [illegible] water.

Serum cholesterol levels were [illegible] (mean 6.57 mmol/l, S.D. 1.34) (Tab[illegible] higher than in a Swedish normal pop[illegible] expected in a group selected for early [illegible] tion. The serum levels of α-lipoprotein [illegible] and triglycerides as well as the cal[illegible] glucose metabolism are shown in Tab[illegible]

The control group consisted of 4[illegible] serum cholesterol and age at the ti[illegible] were selected according to their seru[illegible] among a random population of 30[illegible] males from the Section for Preventive [illegible] undergone physical examination 4–6 [illegible] biopsies. All controls had been inform[illegible] dures beforehand and had given their [illegible] ticipation.

The age distribution of the controls [illegible] to the selection principles used. The [illegible] controls between the five subgroups [illegible] habits was 8, 13, [illegible], 11 and 5, respe[illegible] cholesterol levels of the controls we[illegible] [illegible]ed and are shown together with oth[illegible] [illegible] in Table I.

The skin biopsies were stored for [illegible] volume of Ringer glucose at −70°C [illegible] Immediately after thawing, epiderm[illegible] separated by heating the specimens [illegible] 55°C for 30 sec, followed by careful [illegible] microscope (17). In this way, sw[illegible] and hairs could be removed as well. [illegible] tion level was [illegible] by [illegible]

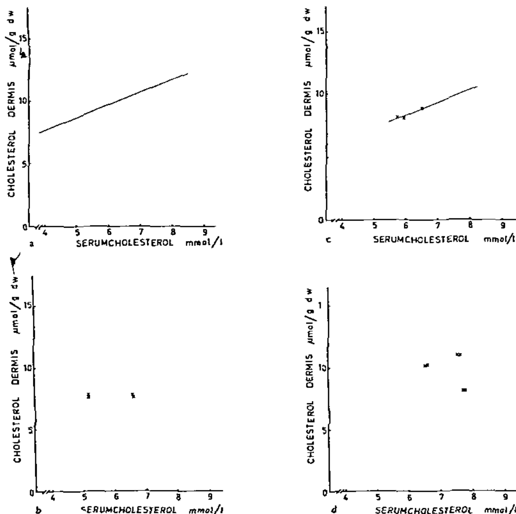



*Fig 4* Cholesterol content in dermis plotted against serum cholesterol (*a*) Non-smoking patients ($r$ +0 64 $n$ 8 2 $p<0$ 01) (*b*) smoking patients ($r$ +0 79 $n$ 16) (*c*) non smoking controls ($r=+0$ 64 $n=21$ ? $p<0$ 01) (*d*) smoking controls ($r=-0$ 08 $n=21$)

## DISCUSSION

The present study was designed to test the hypothesis that the development of atherosclerosis in humans might be paralleled by changes in skin lipid composition The test group consisted of subjects with early myocardial infarction which means that our conclusions are based on the assumption that this group is characterized by an increased degree of atherosclerosis as well In fact it has been stated that patients with myocardial infarction do show an increased degree of atherosclerosis (18)

Boissou et al (1) proposed that an increased degree of atherosclerosis is coupled with an increased skin cholesterol content Similarly Girardet et al (13) found increased skin cholesterol contents in patients with coronary atherosclerosis Our results do *not* show increased skin cholesterol contents in

Table III *DNA (mg/g d w ) in dermis (mean ± S D )*

No of subjects given in parentheses

| | Patients | Controls |
|---|---|---|
| Non smokers | 3 33+0 45 (13) | 3 37+0.58 (19)* |
| Smokers | 3 72±0 74 (11) | 3 85±0 78 (20) |

* Significant difference ($2p<0$ 05)

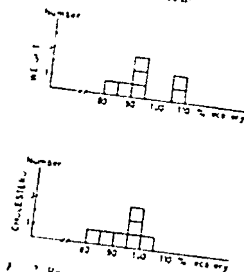



Fig. 2. Recovery determinations for weight and cholesterol.

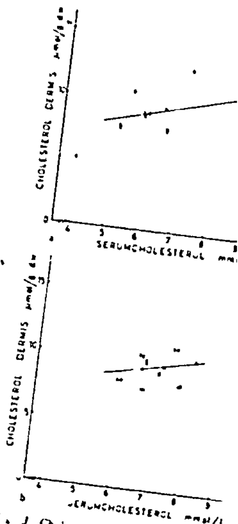



Fig. 3. Cholesterol content in dermis plotted against serum cholesterol. (a) Patients (r = +0.4[illegible], n = [illegible] 0.05). (b) Controls (r = +0.32, n = 42, 2p [illegible] 0.05).

## RESULTS

Cholesterol contents did not differ significantly between patients and controls (Table II). In both groups a significant positive correlation was found between cholesterol content in dermis and in serum (Fig. 3). Cholesterol contents did not differ significantly between non-smokers (subgroups 1 and 2) and smokers (subgroups 3, 4 and 5) (Table II). The positive correlation between dermis and serum cholesterol stated above was found only in non-smokers, not in smokers, both among patients and controls (Fig. 4).

DNA contents did not differ significantly between patients and controls (Table II). DNA contents in dermis were significantly lower in non-smokers than smokers among controls (Table III). A tendency towards a similar difference was found in patients (Table III).

In non-smoking patients a significant positive correlation was found between cholesterol content in epidermis and α-lipoprotein cholesterol concentration in serum. This was not the case in smokers (Fig. 5).

Table II. [illegible] cholesterol and DNA (mean ± S.D.) [illegible] of subjects given in parentheses

| | Patients | Controls | Non-smokers | Smokers |
|---|---|---|---|---|
| Cholesterol (µmol/g d.w.) | | | | |
| Epidermis | 61.9 [illegible] 4 | 66.0 [illegible] (47) | 65.7±5.2 (34) | 64 [illegible] 6.0 [illegible] |
| Dermis | 9.44 1.56 ([illegible]) | 9.[illegible]8 1.34 (42) | 9.31±1.66 (34) | 9.[illegible] 1.4[illegible] |
| DNA (µg/mg d.w.) | | | | |
| Epidermis | 16 [illegible] | 15.5±3.5 (40) | 15.2±2.7 (31) | 6.4 3.[illegible] (11) |
| Dermis | 1.51 0.6[illegible] 4 | 3.61 0.72 (37) | 3.35 0.53 ([illegible]) | 3.[illegible] 2.[illegible] (11) |

[illegible] significant difference (2p [illegible] 0.05)

[illegible]

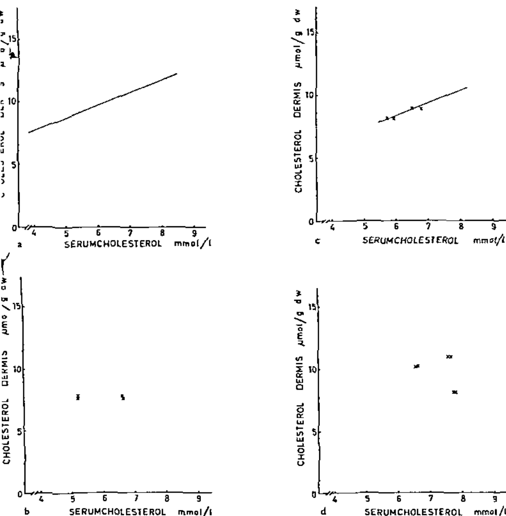



*Fig 4* Cholesterol content in dermis plotted against serum cholesterol (*a*) Non smoking patients ($r=+0\ 64$ $n$ 8 2 $p<0\ 01$) (*b*) smoking patients ($r$ $+0\ 29$ $n=16$) (*c*) non smoking controls ($r=+0\ 64$ $n=21$ 2 $p<0\ 01$) (*d*) smoking controls ($r=-0\ 08$ $n=21$)

## DISCUSSION

The present study was designed to test the hypothesis that the development of atherosclerosis in humans might be paralleled by changes in skin lipid omposition The test group consisted of subjects with early myocardial infarction which means that our conclusions are based on the assumption that this group is characterized by an increased degree of atherosclerosis as well In fact it has been stated that patients with myocardial infarction do show an increased degree of atherosclerosis (18)

Boissou et al (1) proposed that an increased degree of atherosclerosis is coupled with an increased skin cholesterol content Similarly Girardet et al (13) found increased skin cholesterol contents in patients with coronary atherosclerosis Our results do *not* show increased skin cholesterol contents in

Table III *DNA (mg/g d w ) in dermis (mean ± S D )*

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S gnificant difference (2 $p<0\ 05$)

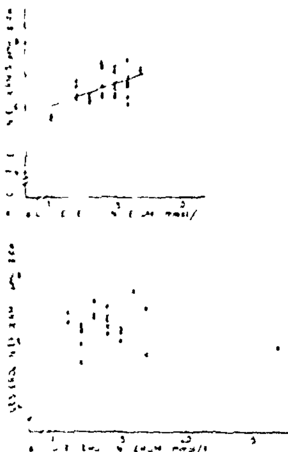


Fig. 4. Cholesterol content in epidermis plotted against cholesterol in serum. (a) Non smokers (r = +0.51 n = 14, P < 0.01) (b) smokers (r = 0.04, n = 31)

patients with early myocardial infarction. We did however find a significant positive correlation between dermis cholesterol content and serum cholesterol. In view of the pronounced vascularization of dermis this might be explained by an entrapment of blood in dermal capillaries. Dermis cholesterol content might therefore to some extent reflect serum cholesterol level. Boissou et al. and Girardet et al. used skin specimens mainly consisting of dermis. It is therefore conceivable that their results reflect differences in serum cholesterol concentration. As our controls were matched for serum cholesterol this would also explain the lack of consistency between our results and theirs.

The positive correlation between dermis cholesterol content and serum cholesterol could only be found in non smokers. both among patients and controls. Nicotine in tobacco smoke induces a pressor amine response, which among other effects may lead to changes in dermal blood flow. It is therefore possible that the amount of blood trapped in dermal capillaries may be more variable in smokers than in non smokers. The contribution of blood cholesterol to dermis cholesterol content would therefore vary in smokers. This might explain the lack of correlation between dermis cholesterol content and serum cholesterol among these.

Skin DNA content did not differ significantly between patients and controls. Dermis DNA content was higher in smokers than in non smokers. This difference was significant for controls but not for patients. At the same time dermis cholesterol content did not differ significantly between smokers and non smokers. This might indicate increased cellularity and decreased cell size in dermis in smokers. This might result from an increased cell turnover with cells of shorter life span.

One might conclude that the determination of cholesterol and DNA in skin biopsies seems to be of limited value in differentiating individuals at risk of myocardial infarction from others. The value is probably also limited in judging the degree of atherosclerosis in an individual. Our data suggest that no extra information is gained compared to a routine serum cholesterol determination together with an assessment of smoking habits. Some differences were found between smokers and non smokers. The significance of these differences remains to be clarified.

## REFERENCES


1. Boissou H, Pieraggi M Th, Julian M, Bouad F, Douste Blazy L, Latorre L & Charlet J P. Identifying atherosclerosis and aortic atheromatous by skin biopsy. Atherosclerosis 19: 449, 1974.
2. Bondjers G & Bjorkerud S. Fluorometric determination of cholesterol and cholesteryl ester in tissue on the nanogram level. Anal Biochem 42: 363, 1971.
3. — Cholesterol content in arterial tissue in relation to serum lipoproteins in man. Artery 2: 200, 1976.
4. Brown M S, Ho Y K & Goldstein J L. The low density lipoprotein pathway in human fibroblasts—relation between cell surface receptor binding and endocytosis of low density lipoproteins. Ann NY Acad Sci 275: 244, 1976.
5. Burstein M & Samaille J. Nouvelle méthode de séparation et de dosage des lipoprotéines de faible densité. Ann Biol Clin 17: 23, 1959.
6. Carlson L A. Determination of serum glycerides. Acta Soc Med Upsalien 64: 208, 1959.
7. Constantinides P. Experimental atherosclerosis. Elsevier, Amsterdam 1965.

8 Cramer K & Isaksson B An evaluation of the Theorell method for the determination of total serum cholesterol Scand J Clin Lab Invest 11 213 1959
9 Curry M D Alaupovic P & Suenram C A Determination of apolipoprotein A and its constitutive A I and A II polypeptides by separate electroimmunoassays Clin Chem 22 315 1976
10 Elmfeldt D Wilhelmsen L Tibblin G Vedin A Wilhelmsson C & Bengtsson C Registration of myocardial infarction in the city of Goteborg Sweden A community study J Chron Dis 28 173 1975
11 Folch J Lees M & Sloane Stanley H H A simple method for the isolation and purification of total lipids from animal tissues J Biol Chem 226 497 1957
12 Freedberg I M Effects of local therapeutic agents upon epidermal macromolecular metabolism J Invest Dermatol 45 529 1965
13 Girardet M Jacotot M Cachera J P & Beaumont J L Cholesterol cutane dans les maladies coronaneures chez l homme Comparaison de deux techniques d extraction Paroi Arterielle 4 59 1977
14 Gordon T Sorlie P & Kannel W B The Framingham study An epidemiological investigation of cardiovascular disease section 27 Coronary heart disease atherothrombotic brain infarction intermittent claudication—a multivariate analysis of some risk factors related to their incidence Framingham study 16 year follow up US Department of Health Education and Welfare 1971
15 Keys A Dietary factors in atherosclerosis In Cowdry s arteriosclerosis (ed H T Blumenthal) p 576 Thomas Springfield 1967
16 Kissane J M & Robins E The fluorometric measurement of deoxyribonucleic acid in animal tissue with special reference to the central nervous system J Biol Chem 233 184 1958
17 Portman O W Esterified fatty acids and cholesterol Adv Lipid Res 8 41 1970
18 Roberts W C The coronary arteries in coronary heart disease morphologic observations In Atherosclerosis and coronary heart disease (ed W Likoff B L Segal W Insull & J H Moyer) p 40 Grune & Stratton New York 1972
19 Wiklund O Fager G Craig I H Wilhelmsson C E Vedin A Olofsson S-O Bondjers G & Wilhelmsen L Alphalipoprotein cholesterol levels in relation to acute myocardial infarction and its risk factors Scand J Clin Lab Invest In press 1979
20 Wiklund O Gustafson A Bergstrand R Vedin A & Wilhelmsson C High density lipoproteins (HDL) in young male myocardial infarction survivors In High density lipoproteins and atherosclerosis (ed A M Gotto N E Miller & M F Oliver) p 127 Elsevier/North Holland Biomedical Press Amsterdam and New York 1978
21 Wilhelmsen L Wedel H & Tibblin G Multivariate analysis of risk factors for coronary heart disease Circulation 48 950 1973

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# Treatment of Dilutional Hyponatremia in Congestive Heart Failure

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ABSTRACT Water restriction is a slow and difficult way to treat dilutional hyponatremia during diuretic therapy of congestive heart failure An i v infusion of 400–1400 mmol hypertonic saline combined with repeated i v injections of loop diuretics was used instead in 9 cases (6 patients) In 4 cases with dominating left heart failure the serum sodium concentration increased and the heart failure was not aggravated as judged from pulmonary rales and body weight Two of the five cases with dominating right heart failure responded in the same favourable way, but body weight increased 1–2 kg and hyponatremia reappeared in three The only difference observed between responders and non responders was that the responders were free from leg edema This treatment of dilutional hyponatremia seems worth further cautious use in situations in which water restriction is troublesome but it should probably be reserved for patients without severe right heart failure

*Key words* hyponatremia congestive heart failure treatment

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During treatment of congestive heart failure with diuretics some patients develop hyponatremia Usually the hyponatremia can be left without treatment but sometimes it gives rise to symptoms and needs therapy

Administration of sodium has been reported to aggravate the clinical state in dilutional hyponatremia associated with congestive heart failure (3 4) but the reports are published before the introduction of loop diuretics The common treatment is water restriction which however is unpleasant to the patient increases the serum sodium slowly and is difficult to maintain for longer periods Therefore we started a cautious treatment with i v infusion of hypertonic saline combined with repeated i v injections of loop diuretics

## PATIENTS AND METHODS

The indications for the saline–diuretic treatment were 1) congestive heart failure but not frank pulmonary edema 2) two serum sodium values below 130 mmol/l (normal range 135–145) 3) a constant and normal serum creatinine value (normal range 0 08–0 13 mmol/l) 4) difficulties in performing water restriction Six hospitalized patients fulfilled these criteria three of them on two occasions giving a total of 9 cases Two patients were females aged 57–79 years and four males 36–72 years Their diagnoses were acute myocardial infarction with moderate left heart failure (case 1) acute myocardial infarction complicated by left ventricular aneurysm and severe left and moderate right heart failure (cases 2 and 3) presbycardia with moderate left heart failure (case 4) severe aortic stenosis (unwilling for surgery) with moderate left and severe right heart failure (cases 5 and 6) congestive cardiomyopathy with moderate left and severe right heart failure (case 7) mb Ebstein with severe right heart failure (case 8+9) All patients had been on diuretics before admission except case 4 who had undergone a transvesicular prostatectomy some days earlier All had stable normal serum creatinine values 0 09–0 11 mmol/l The serum sodium values are presented in Table I

The hypertonic saline infusion was administered during 4–6 hours The infused amount of sodium chloride administered in about 600 ml water was roughly estimated as b wt ×0 7 (135 serum sodium concentration) mmol As a safety measure the first patient received less sodium chloride than calculated Furosemide was administered intravenously during and after the infusion in about the same dose as on the day before Half the peroral dose was considered equivalent to the i v dose (1) In one patient (case 2+3) with severe left heart failure the dosage was increased on the day after the infusion as a safety measure Otherwise cardiac glycosides and peroral diuretics were unchanged during and after the treatment in all patients

The infusion was given in the afternoon or evening of day 1 The lungs were auscultated every morning on days 1–3 and repeatedly during and after the infusion The patients were weighed and the blood samples for serum sodium estimations were collected in the mornings

## RESULTS

*Dominating left heart failure* (cases 1–4)

The serum sodium concentration increased from 117–128 to 131–138 mmol/l No patient became

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Table I *Clinical and laboratory data on the 9 cases with dilutional hyponatremia before and after treatment with i v hypertonic saline infusion and loop diuretics*

| | Day | Case 1 ♀ | Case 2 ♀ | Case 3 ♀ | Case 4 ♂ | Case 5 ♂ | Case 6 ♂ |
|---|---|---|---|---|---|---|---|
| Age (y) | | 79 | 57 | 57 | 72 | 68 | 68 |
| Sodium chloride infused (mmol) | 1 | 400 | 600 | 500 | 800 | 400 | 700 |
| Serum sodium (mmol/l) | 1 | 117 | 124 | 128 | 120 | 127 | 122 |
| | 2 | 131 | 135 | 138 | 135 | 132 | 125 |
| | 3 | 134 | 133 | 138 | – | 130 | 127 |
| Body weight (kg) | 1 | 57 7 | 70 0 | 69 2 | 84 0 | 75 6 | 69 0 |
| | 2 | 56 4 | 68 8 | 67 2 | 84 0 | 76 6 | 67 2 |
| | 3 | 56 2 | – | 66 9 | – | 77 7 | 66 8 |
| Rales[a] | 1 | B | S | S | B | B | B |
| | 2 | B | B | S | B | B | B |
| Diuretics[b] (mg/day) | 1 | F 60 i v | F 220 i v | F 420 i v | F 80 i v | F 330 i v<br>S 50 p o | F 120 i v<br>H 150 p o<br>S 75 p o |
| | 2 | F 40 i v | F 360 i v | F 480 i v<br>F 240 p o | F 40 i v | F 640 p o<br>S 50 p o | F 240 p o<br>H 150 p o<br>S 75 p o |
| Urine (ml/l h) after the same dose of F i v | | | | | | | |
| Before infusion | | – | – | – | 75 | 125 | – |
| During infusion | | – | – | – | 350 | 75 | – |

[a] B = basally S = up to scapula
[b] F = furosemide H = hydrochlorothiazide S = spironolactone B = bumetanide

dyspnoic and the rales did not ascend to a higher level The body weight decreased in three cases and remained unchanged in one One patient (case 2+3) lost 8 kg in weight during the week after the second treatment Case 3 developed hypokalemia (3 2 mmol/l) on day 2

Furosemide diuresis before and during the infusion could be compared in case 4 who had a post operatively inserted bladder catheter Immediately before the infusion 20 mg of i v furosemide was followed by 150 ml urine in two hours and during the last two hours of infusion the diuresis after the same dose of furosemide was 700 ml The interval between the furosemide doses was four hours

*Dominating right heart failure* (cases 5–9)

All five cases were treated with spironolactone for several days before the infusion Serum sodium concentration increased from 118–128 to 125–137 mmol/l in all patients after the infusion In case 6 the level did not reach 130 mmol/l

Cases 5 8 and 9 gained 1 1–2 1 kg in weight in two days after the infusion but did not become dyspnoic and the rales did not increase In all three cases the hyponatremia soon relapsed After two weeks case 5 had lost 6 more kg in weight and his leg edema had disappeared but the hyponatremia persisted He was then treated with another saline infusion (case 6) and this time the weight dropped and the serum sodium value remained higher The patient representing cases 8+9 did never become free from his leg edema Case 7 did not have any leg edema and responded favorably

The voided urine during 4 hours following one and the same dose of furosemide before and during the infusion was measured in the non responding cases 5 and 8 In case 5 the volumes were 500 and 300 ml and in case 8 320 and 650 ml respectively

Irrespectively of left or right heart failure hyponatremic symptoms like lethargy and confusion were reduced or had disappeared after treatment.

## DISCUSSION

When it for various reasons becomes difficult to continue water restriction in dilutional hyponatremia the situation is troublesome The idea

| Case 7 ♂ | Case 8 ♂ | Case 9 ♂ |
|---|---|---|
| 36 | 36 | 36 |
| 1 400 | 500 | 700 |
| 118 | 128 | 127 |
| 137 | 132 | 130 |
| 129 | 132 | – |
| 116 | 85 9 | 78 9 |
| 116 | 88.8 | 81 0 |
| 107 | 87 5 | 80 0 |
| B | 0 | 0 |
| B | 0 | 0 |
| F 80 i v | F 90 i v | F 180 i v |
| H 75 p o | S 75 p o | H 75 p o |
| S 150 p o | | S 75 p o |
| B 4 i v | F 200 p o | F 360 p o |
| F 160 p o | S 75 p o | H 75 p o |
| H 75 p o | | S 75 p o |
| S 150 p o | | |
| – | 80 | – |
| – | 165 | – |

behind treatment with hypertonic saline infusions was that potent simultaneously administered i v diuretics would prevent sodium accumulation and that the excreted saline would remove the water excess This idea was to some extent supported by the finding in the single patient with urinary bladder catheter (case 4) that diuresis following an equal i v dose of furosemide was about five fold during the infusion compared with urine volumes immediately before This patient had left heart failure In two of the three non responders with dominating and severe right heart failure the voided urine volumes were measured with different outcome The diuresis during the infusion was doubled in one but slightly decreased in another case following one and the same dose of furosemide This sodium retention occurred in spite of treatment with spironolactone in both patients but the doses might have been too small (50 and 75 mg/day)

Four of the six patients responded favorably to the treatment Why did case 5+6 not benefit from the first but from the second treatment is a difficult question as the two situations differed in so many respects e g the serum sodium level and the body weight were lower before the second treatment and the total diuretic regime was different Yet it seems likely that *the retention of the first sodium chloride* infusion is in some way coupled to the more edematous state then At that time the patient weighed 6 kg more and had marked leg edema This clinical picture may not be solely the result of secondary hyperaldosteronism during the more active life before hospitalization but when persisting after several weeks in hospital it is also consistent with an ongoing hyperaldosteronism which may partly depend on overtreatment with diuretics (2) Another difference between the two occasions was a higher dose of spironolactone (75 vs 50 mg/day) during the second treatment

The acute risk of treatment with sodium chloride infusion is to provoke pulmonary edema by sodium retention The sodium balance was not followed It was considered safe to control the risk of pulmonary edema by frequent auscultations of the lungs during and after the infusion and the upper level of the rales did not increase in any patient

## CONCLUSION

In the treatment of dilutional hyponatremia hypertonic saline infusion combined with diuretic injections seems to be an alternative to water restriction but for the present it seems wise to avoid treatment of patients with severe right heart failure Close observation of the patient during treatment is however necessary with regard to both aggravation of the heart failure and risk of inducing hypokalemia

## REFERENCES


1 Greither A Goldman S Edelen J S Cohn K & Benet L Z Erratic and incomplete absorption of furosemide in congestive heart failure Am J Cardiol 37 139 1976
2 Hamer J Knight R Miall P Hawkins L A & Dacombe J Plasma aldosterone levels in patients with severe treated heart failure Br Heart J 38 534 1976
3 Orloff J Walser M Kennedy T J & Bartter F C Hyponatremia. Circulation 19 284 1959
4 Weston R E Grossman J Borun E R & Hanenson I B The pathogenesis and treatment of hyponatremia in congestive heart failure Am J Med 25 558 1958

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# Ventricular Arrhythmias and Risk Indicators of Ischemic Heart Disease

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ABSTRACT Three groups of men, 50 with manifest ischemic heart disease (IHD), 50 with risk indicators of IHD and 50 healthy men were examined for ventricular arrhythmias with 24-hour Holter monitoring during normal work and home life Ventricular arrhythmias were most frequent in the IHD intermediate in the risk and least frequent in the control group Multiform ventricular ectopic beats, but no other qualitative characteristic significantly discriminated the three groups The following risk indicators were associated with ventricular arrhythmias ageing, cardiac enlargement and smoking The results suggest that long term ECG monitoring may be of value for the detection of ventricular arrhythmias not only in men with clinical IHD, but also in those with merely risk indicators

*Key words* ischemic heart disease ventricular arrhythmias cardiac enlargement smoking

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An increased number as well as more malignant forms of ventricular ectopic beats (VEBs) have been found to be precursors of both ventricular fibrillation and sudden death in patients with ischemic heart disease (IHD) (9 14 15 21 28) Yet although prognostically useful it remains both difficult and time-consuming to identify patients with IHD who have potentially harmful ventricular ectopic activity To recognize those in the normal population who will experience their first cardiac symptoms with the onset of a fatal attack of ventricular fibrillation is an even more difficult task As has been reported in both American and Swedish studies (2 25 26 29) approximately half of all men who die from IHD before the age of 65—around half of them presenting no previous signs or symptoms of IHD (8 13 20)—die from sudden cardiac death

Conventional risk indicators like hypertension hyperlipidemia and smoking have been found to predict not only the subsequent development of IHD (25) but also in particular sudden cardiac death regardless of whether clinical signs of manifest IHD were present or not (6) Consequently one would expect to observe an increase in potentially harmful cardiac arrhythmias not only in men with manifest IHD but also in those with merely risk indicators In a population study of 10 000 men however no relation was found between coronary risk status and prevalence of VEBs on routine ECG (4) The aim of this study was to investigate by means of 24-hour ECG monitoring whether an increased ventricular irritability is associated with overt IHD alone or also with conventional risk indicators—in the absence of manifest IHD

The following questions were asked 1) Do middle aged men with conventional risk indicators but without clinical evidence of coronary artery disease display more frequent and more malignant forms of ventricular ectopic activity than healthy men? 2) Do the same men show less frequent and less malignant forms than men with symptomatic IHD? 3) Are other clinical characteristics such as hypertension and cardiac enlargement associated with increased ventricular irritability in the absence of evidence of myocardial ischemia?

## PATIENTS AND METHODS

Three groups of 50 men each one with manifest IHD one with conventional risk indicators of IHD and one healthy control group were studied All 150 men were selected from a population of 4 000 men aged 40–65 employed in three large companies with well developed and efficient medical departments in the Stockholm area Regular

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*Abbreviations* IHD = ischemic heart disease VEBs = ventricular ectopic beats MI = myocardial infarction AP = angina pectoris

Table I *Distribution of risk indicators in each group*

| | IHD | | Risk | | Control | |
|---|---|---|---|---|---|---|
| | n | % | n | % | n | % |
| Treatment for hyper tension | 9 | 18 | 43 | 86 | | |
| Treatment for hyper lipidemia | 10 | 20 | 12 | 24 | | |
| Treatment for diabetes | 4 | 8 | 6 | 12 | | |
| Smoking 20 cig /d | 9 | 18 | 9 | 18 | 5 | 10 |

health check ups of all middle aged men including medical history and the assessment of coronary risk indicators were performed in these companies

*IHD group*

All men registered in the medical departments as having myocardial infarction (MI) or angina pectoris (AP) were included A total of 53 men with manifest IHD were found Two refused to participate Of the remaining 51 men 32 had had MI and 19 AP only The clinical history of MI was verified by examination of hospital records Typical enzyme patterns or ECG changes were required for diagnosis The presence of AP was assessed by the London School of Hygiene Questionnaire (24) Typical AP was found both by questionnaire and clinical investigation in four patients A standardized exercise ECG with continuously increasing work load was performed in 15 patients with less typical angina An ischemic ST segment depression of ≥1 mm was required for the diagnosis of IHD In 14 of these patients the presence of IHD was confirmed in one patient it could be ruled out by a repeated exercise test after i v administration of propranolol The mean age at first onset of IHD was 52 8 and at examination 57 9 years Thus an average of 5 1 years had elapsed between onset of IHD and examination

*Risk group*

Fifty men with one or more risk indicators of IHD were obtained from the health screening records They were selected to match individually by age and occupational level the men in the IHD group They were on treatment for hypertension hyperlipidemia or diabetes or smoked 20 cigarettes or more per day The distribution of these characteristics in all groups is shown in Table I Atypical chest pain was reported by 21 subjects in the risk group who were all submitted to the standardized exercise ECG In 15 cases no ST changes were found during exercise Atypical ST changes which were judged by two independent cardiologists not to be caused by ischemia were found in six cases

*Control group*

Fifty men free from IHD or known risk indicators of IHD were selected from the company pay rolls to match individually by age and occupational level the men in both the IHD and the risk group

Four of the 50 originally designated men in both the risk and the control group did not want to participate and had to be replaced by four other men matched in the same way

All men were subjected to a continuous 24 hour ECG recording during normal work and home life An apical sternal lead was applied in which an exploring electrode was placed over the apex an indifferent over the mid sternum and a ground electrode on the right side of the chest (3) The Holter Avionics Electrocardiocorder Model 400 and Avionics Composite Electrocardiscanner Model 650 were used The same thoroughly trained laboratory technician analyzed all tapes All abnormalities were written out on ECG paper and reviewed by the author and an independent cardiologist

All men were in sinus rhythm at the beginning of the recording The following ECG criteria were required for identification of VEBs 1) Configuration different from regular QRS complex 2) Wide complex (≥0 12 sec) 3) No preceding P wave 4) Prematurity and compensatory pause

In addition all men were subjected to the following examination procedures 1) A standard physical examination of the heart lungs and peripheral vessels 2) Systolic

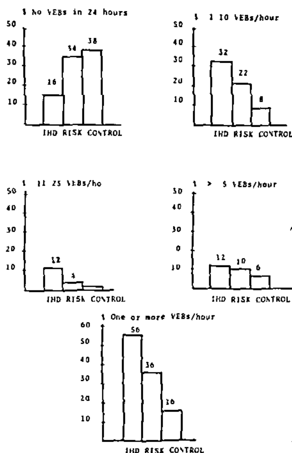



*Fig 1* Incidence of VEBs in the IHD risk and control groups over 24 recorded hours (Percentages of men in each frequency category )

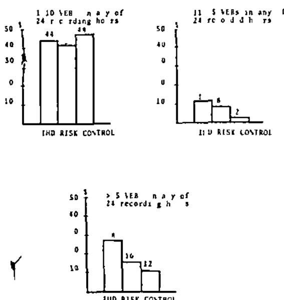



*Fig 2* Incidence of VEBs in the hour of maximal ventricular ectopic activity (Percentage of men in each frequency category)

(phase 1) and diastolic (phase 5) BP was measured in the supine position before and after 15 min rest and in the sitting position The mean of the three measurements was calculated 3) Fasting blood samples were drawn and serum cholesterol triglycerides and glucose were analyzed by standardized chemical laboratory methods 4) Heart size was estimated from a frontal and a sagittal X ray of the chest Cardiac enlargement was defined as a relative heart volume of more than 500 ml borderline enlargement as 451–500 ml/m² BSA 5) Height was expressed in cm weight in kg Relative weight was calculated as weight/height −100 (7) 6) Social status was assessed by the Hollingshead scale (11) 7) An index (5) of past and present smoking including number of cigarettes cigar and pipe smoking was applied 8) The average alcohol consumption during the past ten years was estimated and expressed in grams of absolute alcohol consumed per week 9) Medication (drug and dosage) during the past month was recorded

In order to correlate these characteristics to ventricular irritability an index of ventricular ectopic activity based on the current prognostic classification by Lown et al (14) was formed The average number of VEBs per hour over the 24-hour period was calculated Grade 1=no VEBs during the recording Grade 2=single uniform VEBs only Grade 3=complex ventricular arrhythmia presence of couplets multiform VEBs R-on T phenomenon or ventricular tachycardia

*Statistical methods*

Differences between groups were analyzed by means of the $\chi^2$ test When the number in one of the cells was below ten Fisher's exact probability test was used (1) In order to examine the relationship between ventricular ectopic activity and risk indicators of IHD a stepwise multiple regression analysis was applied Differences between arrhythmia groups were tested by analysis of variance

## RESULTS

The incidence of ventricular ectopic activity in the three groups is shown in Fig 1 Significantly fewer men in the IHD group than in the risk group (Fisher $p=0\ 04$) and the control group ($\chi^2=5\ 07$ $p<0\ 05$) were entirely free from VEBs Significantly more men in the IHD group than in the control group ($\chi^2=8\ 68$ $p<0\ 01$) displayed an average of 1–10 VEBs/hour over the 24-hour period The men in the risk group showed an intermediate frequency higher than but not significantly different from that of the control gorup ($\chi^2=2\ 08$) No significant differences between the groups were found when only those who had an average of 11–25 VEBs/hour or those who had more than 25 VEBs/hour were included The most pronounced difference between men with IHD and healthy men was demonstrated when one or more VEBs/hour was chosen as cut-off point ($\chi^2=33\ 86$ $p<0\ 0001$) Significantly more men

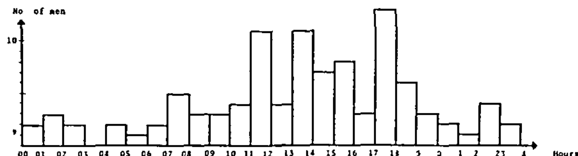



*Fig 3* Distribution of maximal incidence of VEBs over 24 recorded hours in the total sample

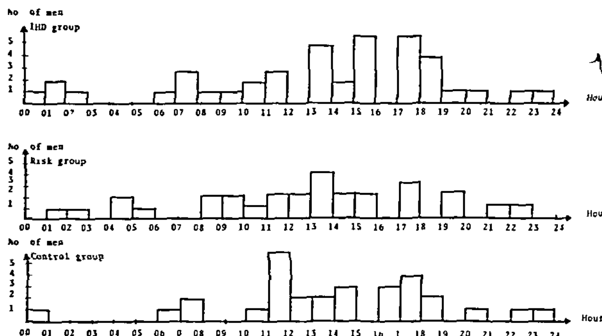



*Fig 4* Distribution of maximal incidence of VEBs over 24 recorded hours in the three groups of men

with risk indicators than healthy men were also found in this category ($\chi^2=4\ 21$ $p<0\ 05$) but compared to the men with IHD they were significantly fewer ($\chi^2=14\ 65$ $p<0\ 001$)

The incidence of VEBs in each man in each hour was also correlated to the mean heart rate in each hour When the effects of heart rates were corrected for and the frequency categories of Fig 1 were applied the proportions between groups were approximately the same as before correcting for heart rates

When the hour with the maximum incidence of ventricular ectopic activity was analyzed for each man a similar tendency was found (Fig 2) Of all men with more than 25 VEBs at this hour those in the IHD group had more VEBs than those in the risk group who in their turn had more VEBs than the controls although the differences did not quite reach statistical significance

The highest number of VEBs registered in any of the 24 recorded hours in any man with IHD was 510 in any man with risk indicators 560 and in any healthy man 260 The distribution of maximal incidence of VEBs over 24 recorded hours is shown in Fig 3 In the majority of the total sample there was an increase in VEBs during the day between 7 a m and 7 p m and a decrease at night from 11 p m to a m

When the three groups were compared certai differences in distributions were observed (Fig 4) Few men in the control group had their maxima incidence of VEBs at night and none between 1 an 6 a m The most frequent peak hour in this grou was before noon Five men in the risk group an five in the IHD group had their maximal incidenc of VEBs at night (11 p m –6 a m ) In the ris group the distribution was quite regular over the 2 hours in the IHD group the hours of maximal inci dence were most frequently found in the afternoon

Qualitative characteristics of VEBs are shown i Fig 5 Only multiform VEBs were significantl more common in men with IHD than in healthy me (Fisher $p=0\ 03$) Couplets of VEBs and ventricula tachycardia were also most common in the IH group but so infrequent that no statistically signifi cant differences appeared Nor could the R on T phenomenon bigeminy or trigeminy significantly differentiate the three groups The finding of complex ventricular arrhythmias was more common in men with higher incidence of VEBs All except four of those who showed more than 25 VEBs/hour also had complex ventricular arrhythmia

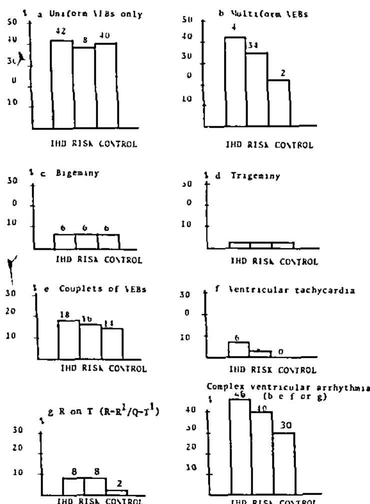



*Fig 5* Qualitative assessment of VEBs in the IHD risk and control groups (Percentages of men with a characteristic)

The prognostic grading of ventricular ectopic activity described above was related to a number of conventional risk indicators for IHD by means of a stepwise multiple regression analysis Systolic and diastolic BP serum cholesterol triglycerides fasting glucose roentgenologic cardiac size relative body weight smoking alcohol consumption social status and age were included In order to account for the possible confounding effect of the presence of IHD on the significance of these variables for ventricular arrhythmias the division into IHD risk and control group was also included in the multivariate analysis Three variables were found to significantly discriminate the arrhythmia grades old age ($p<0.001$) excessive smoking ($p<0.05$) and cardiac enlargement ($p<0.05$) When the significance of these three variables was analyzed separately in each group a similar pattern was found only in the risk group In the control group the incidence of VEBs was associated only with old age and in the IHD group with none of these variables

To account for possible effects on arrhythmias current medication was analyzed in each group $\beta$ Blocking agents were equally common in the IHD and in the risk groups (48 and 46%) but were not administered in the control group Twenty per cent of the men with IHD were on digitalis therapy 2% of the men in the risk group and none of the controls No other medication with known effects on arrhythmias was reported

## DISCUSSION

Different ways of assessing the incidence of arrhythmia will yield very different results The

length of the recorded period is of great importance as well as the external conditions under which the recording takes place A 24-hour monitoring period was chosen in this study as has been proposed by Lown et al (14) Recording during normal work and home life was used because it was assumed to yield more representative data about real life conditions" than recording for example in a hospital setting

The finding of this study that 84% of men with IHD have some form of ventricular ectopic activity is in good accordance with other reports of 24-hour ECG recordings (14) In contrast to the findings of Crow et al (4), in this study also men with risk indicators who showed no signs or symptoms of *IHD displayed more VEBs than healthy men* The finding of highest frequencies of VEBs in the IHD group intermediate in the risk group and lowest in the control group was maintained for all frequencies of VEBs except for those who had only occasional VEBs (10 or less in the hour of maximal incidence of VEBs)

The distribution of ventricular ectopic activity over the diurnal period is not even (16 17) Almost half of all men with VEBs had them during 6 of the 24 recorded hours (31% of the IHD 52% of the risk 61% of the control gorup) This may also explain why in studies using varying lengths of ECG recording the proportion of men with IHD who show ventricular ectopic activity varies from 10 to 90% (14) Ventricular ectopic activity increased during the day with a peak around noon When compared with other circadian variations the similarity of variation in ventricular arrhythmia with cathecolamine excretion curves is striking Such excretion also reaches a peak in the early mid-day and is extended into the afternoon as shown by Theorell and Åkerstedt (27) among others

When the groups were analyzed separately certain differences became visible In the control group the mid-day peak of ventricular arrhythmias was most pronounced No man had his maximal incidence of VEBs between 1 and 6 a m In the risk group the peaks of ventricular ectopic activity were found at any time also at night In the IHD group the maximum was most commonly found in the afternoon but peaks were also found at night. This pattern seems to indicate that ventricular ectopic activity in the healthy control group may be caused mainly by increased sympathetic activity as suggested by Lown et al (17) and consequently occur during the day In the IHD and risk groups the arrhythmias may in addition be due to pathologic changes in the myocardium It has been argued that sudden death is more common at night than could be explained by the incidence of VEBs alone The theory of VEBs caused by different mechanisms may possibly yield an explanation of this discrepancy

Of complex ventricular arrhythmias only multifocality differed significantly between groups with the highest proportion (42%) in the IHD group Reports of multiform VEBs differ from 20% (18) 25% (14) 30% (23) to 36% (19) among patients with IHD and to as much as 34% of a normal *population (10) These discrepancies could be due* to e g differences in length and external conditions of the recording Two of these studies were performed in hospital settings and for only six hours Other reasons for differences are varying age and stages of IHD in the samples studied In the present study the men with IHD had experienced their first symptoms of AP or MI on an average 5 1 years before examination

Finally the significance of clinical characteristics age smoking and alcohol consumption for ventricular arrhythmias was examined As in other studies (4 23) ventricular irritability was found to increase with increasing age Not unexpectedly ventricular arrhythmias were also more frequent and more malignant in men with enlarged hearts and a history of smoking (12) Old age cardiac enlargement and smoking were equally common in the IHD and risk groups but of importance for the occurrence of ventricular arrhythmias only in the risk group Subjects were included in this group only if they were free from myocardial ischemia on exercise ECG These results suggest that once myocardial ischemia is present it seems to be the major pathogenetic mechanism of ventricular arrhythmias But in absence of clinically detectable IHD ageing cardiac enlargement and smoking may be precursors of such arrhythmias

For clinical use the present findings indicate that long term ECG monitoring for arrhythmias not only in men with known IHD but also in those with merely risk indicators may be useful for the detection of candidates for future sudden cardiac death and thus enable therapeutic and preventive intervention

ACKNOWLEDGEMENTS

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## REFERENCES

1 Armitage P Statistical methods in medical research Blackwell Scientific Publications Oxford 1973
2 Biorck G Epidemiology of ischemic heart disease and medical care planning Acta Soc Med Scand 2–3 95 1972
3 Chung E K Principles of cardiac arrhythmias Williams and Wilkins Baltimore 1977
4 Crow R S Prineas R T Dias V Taylor H L Jacobs D & Blackburn H Ventricular premature beats in a population sample Frequency and associations with coronary risk characteristics Circulation (Suppl) III 211 1975
5 Dyer A R Stamler J Bergson D M & Lindberg H A Relationship of relative body weight and body mass index to 14-year mortality in the Chicago Peoples Gas Company study J Chron Dis 28 109 1975
6 Friedman G D Klatsky A L & Siegelaub A B Predictors of sudden cardiac death Circulation (Suppl) III 164 1975
7 Frisk A R Werko L Holmgren A & Strom G Stockholm s City Health Survey 1954 Acta Med Scand 163 1 1959
8 Fulton M Julian D G & Oliver M F Sudden death and myocardial infarction Circulation (Suppl) IV 182 1969
9 Hinkle L E Carver S T & Argyros D C The prognostic significance of ventricular premature contractions in healthy people and in people with coronary heart disease Acta Cardiol (Suppl) 18 5 1974
10 Hinkle L E Carver S T & Stevens M The frequency of asymptomatic disturbances of cardiac rhythm and conduction in middle aged men Am J Cardiol 24 629 1969
11 Hollingshead A B & Redlich R C Social class and mental illness Wiley New York 1958
12 Kannel W B Doyle J T McNamara P M Quickenton P & Gordon T Precursors of sudden coronary death Factors related to the incidence of sudden death Circulation 51 606 1975
13 Kuller L Lilienfeld A & Fisher R Epidemiological study of sudden and unexpected death due to arteriosclerotic heart disease Circulation 34 1056 1966
14 Lown B Calvert A F Armington R & Ryan M Monitoring for serious arrhythmias and high risk of sudden death Circulation (Suppl) III 189 1975
15 Lown B Fakhro A M Hood W B & Thorn G The coronary care unit New perspectives and directions JAMA 199 188 1967
16 Lown B Tykocinski M Garfein A & Brooks P Hemodynamic changes during sleep J Appl Physiol 22 867 1973
17 — Sleep and ventricular premature beats Circulation 48 691 1973
18 Moss A J Schnitzler R Green R & De Camilla J Ventricular arrhythmias three weeks after acute myocardial infarction Ann Intern Med 75 837 1971
19 Myburgh D P & Van Geider A L The nature of ventricular ectopic beats in chronic ischaemic heart disease S Afr Med J 48 1067 1974
20 Myerburg R J & Davis J H The medical ecology of medical safety I Sudden death due to coronary heart disease Am Heart J 68 586 1964
21 Oliver G C Nolle F M Tiefenbrunn J A Kleiger R E Martin T F Krone R J Miller P & Cox J R Jr Ventricular arrhythmias associated with sudden death in survivors of acute myocardial infarction Am J Cardiol 33 160 1974
22 Rehnqvist N Ventricular arrhythmias prior to discharge after acute myocardial infarction Eur J Cardiol 4 63 1976
23 — Ventricular arrhythmias after an acute myocardial infarction Prognostic weight and natural history Eur J Cardiol 7 169 1978
24 Rose G A & Blackburn H Cardiovascular survey methods WHO Monogr Ser 56 1 1968
25 Stamler J Primary prevention of sudden coronary death Circulation (Suppl) III 258 1975
26 The coronary Drug Project Research Group Prognostic importance of premature beats following myocardial infarction Experience in the coronary drug project JAMA 223 1116 1973
27 Theorell T & Åkerstedt T Day and night work Changes in cholesterol uric acid glucose and potassium in serum and in circadian patterns of urinary catecholamine excretion Acta Med Scand 200 47 1976
28 Van Durme J P & Pannier R H Prognostic significance of ventricular dysrhythmias one year after myocardial infarction Am J Cardiol 37 178 1976
29 Wikland B Medically unattended fatal cases of ischemic heart disease in a defined population Acta Med Scand (Suppl) 524 1971

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# The Effect of Quinidine on Digoxin Kinetics in Cardiac Patients

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**ABSTRACT** The pharmacokinetics of digoxin was studied in 11 subjects before and during quinidine treatment Renal clearances of digoxin and creatinine were calculated from plasma concentrations and urinary excretions of digoxin and creatinine in subjects on long term digoxin treatment The investigations were repeated in the same subjects during administration of quinidine Renal clearance of digoxin decreased while plasma concentration of digoxin and renal excretion of digoxin increased in the presence of quinidine, indicating substantial changes of digoxin kinetics, induced by quinidine The reduction in renal clearance of digoxin may be due to a specific inhibition of tubular secretion of digoxin The considerable rise of the plasma digoxin level and the increased excretion of digoxin support the assumption of a major extrarenal mechanism of interaction

*Key words* digoxin digoxin kinetics digoxin intoxication digoxin–quinidine interaction

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In man digoxin is eliminated predominantly by the kidneys leaving only 10–20% of bioavailable digoxin for hepatic elimination The mechanisms of renal elimination are partly glomerular filtration partly tubular secretion The latter pathway being an active secretory process and contributing about 40–60% of total renal excretion of digoxin is a potential site of drug interaction

Recent investigations have shown a two to threefold increase in plasma concentration of digoxin during concurrent administration of digoxin and quinidine Studies by Hager et al (3) and Hooymans and Merkus (4) have demonstrated a reduction in volume of distribution and renal clearance of digoxin during quinidine administration However the pharmacokinetic basis and the clinical implications of this important drug interaction are still insufficiently resolved

This study was performed in order to evaluate the effect of quinidine on renal elimination of digoxin and to assess the clinical significance of the digoxin–quinidine interaction

## STUDY POPULATION AND METHODS

*Subject characteristics* The study comprised 11 patients admitted to the Medical Department of Vejle Hospital for evaluation or control of arteriosclerotic or rheumatic heart disease All were on long term digoxin treatment for paroxysmal atrial fibrillation or congestive heart failure Based upon clinical examination roentgenographic and ECG findings each subject was assessed haemodynamically and classified according to the guidelines of the NYHA Clinical assessment was repeated regularly during the study and showed stable haemodynamic conditions in all subjects

Quinidine therapy was initiated in order to control various arrhythmias Daily ECGs however showed no haemodynamically significant alterations of heart rhythm during the study except for two subjects who developed digoxin intoxication The individual characteristics are summarized in Table I

*Protocol* Cooperative hospitalized patients who had been receiving digoxin for more than four weeks and who exhibited stable haemodynamic conditions were selected for the study Informed consent was obtained in each case before entry Digoxin was administered orally in two daily doses at 7 a m and 5 p m In addition to digoxin and quinidine the following drugs were given bumetanide (pats 3–8 10 11) hydroflumethiazide (pats 1 2) potassium chloride (pats 1 2 4 8 10 11) amiloride (pats 3 7) prednisolone (pat 11) insulin (pat 2) The dose and dose interval of these drugs were fixed during the study In order to fulfil these conditions and to ensure the presence of a steady state equilibrium concerning digoxin each patient was observed for one week

When the study was designed the available information about digoxin–quinidine interaction did not provide a reliable prediction of the rise in plasma digoxin concentration We decided to administer quinidine for ten days with regular assessment of plasma digoxin daily ECG and careful attention to clinical symptoms of digoxin intoxication If a patient showed a rise in plasma digoxin concentration with a concomitant risk of intoxication quinidine

Table I *Subject characteristics*

AHD = arteriosclerotic heart disease RHD = rheumatic heart disease PVC = premature ventricular contractions PAF = paroxysmal atrial fibrillation

| Subj no | Age (y ) | Weight (kg) | Heart disease | Arrhythmia | Degree of heart failure | Digoxin dose (mg/d ) | Quinidine dose (mg/d ) |
|---|---|---|---|---|---|---|---|
| 1 | 82 | 58 0 | AHD | PVC | Mild | 0 125 | 1 000 |
| 2 | 55 | 52 8 | AHD | PVC | Latent | 0 125 | 600 |
| 3 | 59 | 78 0 | AHD | PVC | Mild | 0 125 | 600 |
| 4 | 78 | 80 0 | AHD | PVC | Mild | 0 125 | 1 200 |
| 5 | 51 | 95 0 | AHD | PVC | Mild | 0 3125 | 600 |
| 6 | 54 | 67 2 | AHD | PVC | Latent | 0 1875 | 800 |
| 7 | 65 | 63 5 | RHD | PAF | Mild | 0 125 | 800 |
| 8 | 74 | 64 0 | AHD | PVC | Moderate | 0 250 | 600 |
| 9 | 76 | 54 5 | AHD | PAF | Latent | 0 125 | 1 000 |
| 10 | 89 | 57 0 | AHD | PVC | Moderate | 0 125 | 1 200 |
| 11 | 60 | 56 0 | AHD | PVC | Moderate | 0 250 | 800 |

treatment should be stopped The bias entering the study by this inevitable procedure of exclusion may cause some underestimation of the quinidine induced changes

Before initiation of quinidine therapy two successive 24 hour urine collections and two mid urine blood samples were obtained and assayed for digoxin and creatinine Blood samples were taken nine hours after the last dose of digoxin Quinidine was administered orally in two daily doses at 7 a m and 5 p m After three and eight days the collections of two successive 24 hour urine samples and two mid-urine blood samples were repeated and assayed for digoxin and creatinine

*Data analysis* Renal clearance of digoxin was calculated from corresponding plasma concentration and urinary excretion on the basis of pooled data from each 48 hour urine collection Renal clearance of creatinine was calculated analogously and expressed as mean of all measurements In order to eliminate the influence of inaccurate collection of urine total excretion of creatinine was used as a corrective factor in the calculation of renal excretion of digoxin

Statistical analysis was performed according to Wilcoxon s rank sum test for paired data

*Analytic methods* Plasma and urinary concentrations of digoxin were determined by a radioimmunoassay technique measuring both protein bound and free digoxin (8) Plasma and urinary concentrations of creatinine were determined by a specific ion exchange method (13)

## RESULTS

Eleven subjects entered the study and nine completed ten days of quinidine treatment as prescribed by the protocol Subject 8 did not join the scheduled investigations after five days due to lack of cooperation Subjects 5 and 6 left the study prematurely after five days of quinidine treatment because of an abrupt rise of plasma digoxin concentration Subjects 8 and 10 developed symptoms of digoxin intoxication after 10 and 11 days with peak plasma concentrations of digoxin of 4 75 and 3 75 nmol/l respectively In both cases the intoxication subsided within a few days after digoxin withdrawal and no specific treatment was required

Table II summarizes the pharmacokinetic values obtained All subjects presented an increase in plasma digoxin concentration during quinidine treatment as compared to prequinidine level but the degree and speed of the elevation showed pronounced variation The mean increases after four and nine days were 64 5% ($p<0$ 01) and 107 1% ($p<0$ 01) respectively The most dramatic rise of the plasma digoxin concentration was observed in subjects receiving a relatively high maintenance dose of digoxin or showing relatively advanced congestive heart failure

Renal clearance of digoxin decreased during quinidine treatment except for subject 1 who showed a slight increase The mean reductions after four and nine days were 26 6% ($p<0$ 05) and 28 3% ($p<0$ 01) respectively Our data provided information for a threefold estimation of renal clearance of creatinine in each subject These measurements showed only minor day to-day variations and no significant difference in the absence or presence of quinidine was observed indicating that glomerular filtration rate (GFR) was constant during the study

## DISCUSSION

The pharmacokinetics of digoxin is known from an extensive number of investigations The mechanisms of renal elimination are passive

Table II *Renal clearances of creatinine and digoxin and plasma concentration of digoxin before and during quinidine treatment*

Con = control $Quin_I$ = after four days of quinidine treatment $Quin_{II}$ = after nine days of quinidine treatment

| Subj no | Creatinine clearance (ml/min) | Plasma digoxin (nmol/l) | | | | Digoxin clearance (ml/min) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Con | $Quin_I$ | Con | $Quin_{II}$ | Con | $Quin_I$ | Con | $Quin_{II}$ |
| 1 | 55.2 | 0.81 | 1.06 | 0.81 | 1.31 | 62.0 | 73.4 | 62.0 | 69.5 |
| 2 | 119.8 | 0.69 | 1.00 | 0.69 | 1.13 | 118.0 | 146.0 | 118.0 | 110.0 |
| 3 | 94.6 | 0.63 | 0.88 | 0.63 | 1.38 | 71.0 | 35.4 | 71.0 | 57.4 |
| 4 | 73.5 | 0.88 | 1.00 | 0.88 | 1.00 | 95.0 | 81.4 | 95.0 | 67.4 |
| 5 | 198.3 | 0.88 | 2.00 | – | – | 222.7 | 105.8 | – | – |
| 6 | 149.0 | 0.63 | 1.38 | – | – | 131.7 | 82.5 | – | – |
| 7 | 74.6 | 0.75 | 0.81 | 0.75 | 1.81 | 61.3 | 64.7 | 61.3 | 41.0 |
| 8 | 70.5 | – | – | 1.44 | 4.56 | – | – | 67.6 | 47.4 |
| 9 | 92.5 | 0.63 | 1.31 | 0.63 | 1.38 | 124.2 | 80.8 | 124.2 | 52.3 |
| 10 | 90.1 | 1.00 | 1.75 | 1.00 | 2.31 | 98.3 | 63.5 | 98.3 | 56.8 |
| 11 | 55.9 | 2.63 | 4.50 | 2.63 | 4.75 | 64.0 | 36.0 | 64.0 | 43.5 |
| Mean | 97.6 | 0.95 | 1.56 | 1.05 | 2.18 | 104.8 | 76.9 | 84.6 | 60.6 |
| *p* | | | <0.01 | | <0.01 | | <0.05 | | <0.01 |

glomerular filtration of free digoxin and active tubular secretion of both free and protein bound digoxin The site and character of the tubular transport system is unknown but like other tubular secretory processes the digoxin carrier system is assumed to be limited by a maximum transport capacity In man the level of plasma digoxin corresponding to a saturation of this carrier system has not been identified but it seems to lie far above therapeutic plasma concentrations Several studies have demonstrated a substantial reduction of renal clearance of digoxin during administration of spironolactone suggesting an inhibition of tubular secretion (9 14) The interaction may be due to a close structural similarity between digoxin and spironolactone causing specific inhibition at the receptor site However preliminary investigations have shown similar changes induced by triamterene amiloride and hypokalaemia (10 11) supporting the possibility of a non specific inhibition of the digoxin carrier system Renal elimination of digoxin seems to be unaffected by verapamil (7)

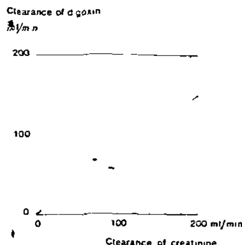



*Fig 1* Relationship between simultaneously measured clearances of digoxin and creatinine before (□) and after nine days (■) of quinidine treatment

Recent investigations dealing with patients on long term digoxin therapy have shown a two to threefold increase in plasma concentration of digoxin in patients receiving simultaneous quinidine treatment as compared to control groups (1 2 4 6) Hooymans and Merkus (4) have demonstrated a mean reduction of 37% in renal clearance of digoxin in three patients during quinidine therapy as compared to values in the absence of quinidine Similarly pharmacokinetic studies by Hager et al (3) have revealed a mean reduction of 30% in renal clearance of digoxin in the presence of quinidine In both studies simultaneous measurements of creatinine clearance showed stable values suggesting a constant GFR and consequently the reduction of renal clearance of digoxin was assumed to be due to a suppression of tubular secretion of digoxin Further pharmacokinetic data from the study by Hager et al have demonstrated a mean reduction of 32% in the distribution volume of digoxin in the presence of

quinidine Consistent with this observation Straub et al (12) have reported a reduced number of myocardial binding sites of digitalis in the presence of quinidine which may reflect a general depletion of digitalis binding sites The degree of plasma protein binding of digoxin is not significantly influenced by quinidine (11)

The results of our study support the assumption of a combined renal/extrarenal mechanism of interaction In agreement with Hager et al (3) and Hooymans and Merkus (4) we observed a significant reduction in renal clearance of digoxin along with stable values of simultaneously measured clearance of creatinine These changes indicate a suppressed tubular secretion of digoxin caused by either a direct inhibitory effect of quinidine or a saturation of the digoxin transport capacity The latter possibility was given consideration and a supplementary steady state study was performed on subjects 7 and 11 after reduction of the digoxin maintenance dose by 50% This showed a reduction of plasma digoxin concentrations to approximately prequinidine levels while clearance of digoxin remained unaltered Thus the suppression of tubular secretion of digoxin seems to be unaffected by the plasma digoxin level apparently eliminating the possibility of a saturation of the secretory capacity

In agreement with the findings of other authors a close linear relationship between clearances of digoxin and creatinine was observed as outlined in Fig 1 The digoxin/creatinine clearance ratio declined from 1 04 in the absence of quinidine to 0 75 after nine days of quinidine treatment By applying the formula of Lavender et al (5) which describes the relationship between clearance of creatinine and GFR at different levels of GFR and by making allowance for an assumed 25% protein binding of digoxin a rough estimate of GFR and free digoxin clearance in each subject is possible The tubular contribution of total renal excretion of digoxin obtained from these data showed a significant decline from 48.5% in the absence of quinidine to 29 8% after nine days of quinidine treatment ($p = 0\ 01$)

Assuming that inhibition of tubular secretion of digoxin is the only mechanism of interaction a rapid reduction of urinary excretion of digoxin followed by a steady rise would be expected in the presence of quinidine A new steady state equilibrium would be characterized by a total renal excretion of digoxin reaching prequinidine level and a plasma digoxin concentration exceeding prequinidine level in proportion to the reduction of renal clearance of digoxin Our data on the contrary showed an increase in urinary excretion of digoxin compared with prequinidine levels with mean values after four and nine days of 16 3% ($p<0\ 05$) and 34 2% ($p<0\ 025$) respectively Furthermore the mean rise of plasma digoxin concentration after nine days corresponds to an increase in total body content of digoxin of about 0 60 mg while the calculated cumulative retention of digoxin is approximately 0 15 mg These observations clearly indicate a major extrarenal mechanism of interaction A reduction in the distribution volume of digoxin seems to be the only mechanism providing a pharmacokinetic basis for the considerable changes in digoxin kinetics observed An impaired hepatic elimination may be a contributing factor Bioavailability of digoxin has not been assessed in this study but the investigation by Hager et al (3) using an intravenous route rules out any significant interaction at the site of absorption

In concordance with previous observations (2 6) our results emphasize the clinical importance of the digoxin–quinidine interaction and oppose the assumption of an altered concentration–response relation as suggested by the findings of Straub et al (12) We must conclude that quinidine induces substantial changes in digoxin kinetics and that the use of these drugs in combination is impeded by a serious risk of digoxin intoxication Until further pharmacokinetic information is available simultaneous administration of digoxin and quinidine deserves careful clinical attention regular assessment of plasma digoxin and should be preceded by a 50% reduction of digoxin loading and maintenance doses

## REFERENCES

1 Doering W & Koenig E The influence of quinidine on serum digoxin concentrations Med Klin 73 1085 1978
2 Ejvinsson G Effect of quinidine on plasma concentrations of digoxin Br Med J 1 279 1978
3 Hager P W Fenster P Mayersohn M Perrier D Graves P Marcus F I & Goldman S Digoxin–quinidine interaction Pharmacokinetic evaluation N Engl J Med 300 1238 1979
4 Hooymans P M & Merkus F W H M Effect of quinidine on plasma concentration of digoxin Br Med J 2 1022 1978
5 Lavender S Hilton P J & Jones N F The measurement of glomerular filtration rate in renal disease Lancet 2 1216 1969

6 Leahey E B Jr Reiffel J A Drusin R E Heissenbuttel R H Lovejoy W P & Bigger J B Jr *Interaction between quinidine and digoxin* JAMA 240 533 1978
7 Pedersen K E & Hvidt S Unpublished data 1979
8 Smith T W Butler V P & Haber E Determination of therapeutic and toxic serum digoxin concentrations by radioimmunoassay N Engl J Med 281 1212 1969
9 Steiness E Renal tubular secretion of digoxin Circulation 50 103 1974
10 — *Suppression of renal digoxin excretion in hypokalemic patients* Clin Pharmacol Ther 23 511 1978
11 — Personal communication 1979
12 Straub K D Kane J J & Bisset J K Alteration *of digitalis binding by quinidine a mechanism of digitalis–quinidine* interaction Circulation (Suppl) 2 II 1978
13 Vedsø S Rud C & Place J F Routine creatinine determination An equilibrium technique involving reusable cation exchange membranes Scand J Clin Lab Invest 34 275 1974
14 Waldorff S Andersen J D Heeboll Nielsen N Nielsen O G Moltke E Sørensen U & Steiness E *Spironolactone-induced changes in digoxin kinetics* Clin Pharmacol Ther 24 162 1978

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# A Familial Syndrome with von Recklinghausen's Neurofibromatosis, Gammopathy and Aorta Outflow Obstruction

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ABSTRACT Three cases of von Recklinghausen's disease have been observed in the same family comprising four members Two of the patients, father and son, had aorta outflow obstruction and biclonal gammopathy (IgG(κ/λ) and IgA(κ)/IgG(κ)) In one of these patients, no concanavalin suppressor cell activity was demonstrated, indicating that the gammopathy may be related to suppressor T cell deficiency Further study of the other family lineages showed that aorta stenosis/mors subita occurred frequently but genetic marker studies failed to reveal any linkage between these entities The syndrome, which has not been reported previously, was probably restricted to the first kindred studied Detailed biochemical and immunological studies of the monoclonal components involved are in progress

*Key words* aorta stenosis gammopathy M-component Recklinghausen s disease suppressor cell activity

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In a recent study (44) we reported on a case with von Recklinghausen s neurofibromatosis aorta stenosis and monoclonal gammopathy During three years of observation the patient developed biclonal gammopathy of IgG(kappa) and IgG(lambda) specificities By accident this patient s son was admitted to our hospital because of a leg fracture and during his stay in the hospital we observed that also he had von Recklinghausen s neurofibromatosis aorta outflow obstruction and biclonal gammopathy

It is well known that von Recklinghausen s neurofibromatosis can be associated with a number of other diseases Interestingly hypertrophic subaortic stenosis has been reported in von Recklinghausen s disease while an association with gammopathy has not been reported before Disturbances in the helper/suppressor systems may explain the appearance of monoclonal components or hypergammaglobulinemia (14) and assay systems for these cells have recently been developed (14) This led us into more detailed clinical genetic and immunological studies of the family which are reported in this communication

## SUBJECTS

Altogether 38 patients and/or their records have been examined The pedigree chart of the kindreds involved is presented in Fig 1 All individuals in generations I and II are dead I 2 and II 1 of heart disease (mors subita) In generation III all deaths were due to heart disease presumably aorta stenosis/sudden death Age at death is indicated in Fig 1 Only one of them (II 1) has been examined alive in detail He had had both von Recklinghausen s neurofibromatosis and gammopathy as well as cardiologically verified aortic outflow obstruction His case history will be outlined in detail in the subsequent text

In generation IV patient IV 1 has aortic outflow obstruction (verified by cardiologist) von Recklinghausen s neurofibromatosis and gammopathy His case history will be outlined in detail in the subsequent text Patient IV 2 had von Recklinghausen s neurofibromatosis and died from a cerebellar tumour 17 years old The records of this patient do not contain information on any heart disease Patient IV 3 died suddenly presumably of aortic stenosis 37 years old No information on eventual gammopathy or von Rechklinghausen s disease exists Patients IV 7 and IV 10 have gammopathy (polyclonal) but are otherwise healthy and have no symptoms from the heart or no signs of von Recklinghausen s disease

In generation V (subjects aged 7–21) none of the individuals have developed manifest heart disease or any physical signs suggesting aorta outflow obstruction Patients V 3 (21 years old) and V 9 (7 years old) however have polyclonal hypergammaglobulinemia Patient V 9 also has atypical neurologic symptoms although he has no classical von Recklinghausen s disease

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*Abbreviations* Hp = haptoglobin Gc = group-specific protein C6 = complement 6 Gm = genetic marker PWM = pokeweed mitogen PPD = purified protein derivative PHA = phytohemagglutinin ORBC = ox red blood cells Con A = concanavalin A FCS = fetal calf serum

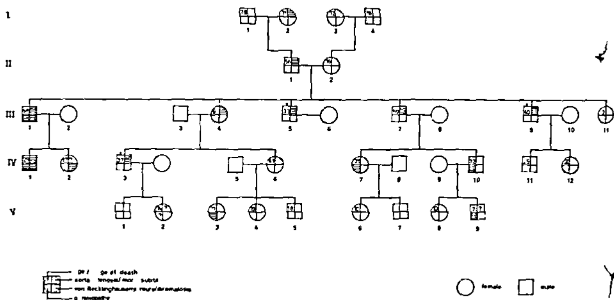


*Fig 1* Pedigree of the kindreds involved

## CASE REPORTS

*Patient III 1*

A 68-year-old man who had noticed skin tumors melanotic skin macules and axillary freckling since the age of 35 No knowledge of similar defects in parents or siblings but his son (patient IV 1 now aged 29 see below) has a similar "disease For the last 2 years the patient has complained of dyspnea during exercise Physical examination demonstrated a rough systolic murmur grade IV–V with radiation to the carotides indicating a stenotic aortic valve The diagnosis was subsequently confirmed by a cardiologist A moderate hepatomegaly was disclosed no overt edema tendency Extensive Dupuytren s contractures were observed in both hands No muskuloskeletal tenderness back pain or other signs suggesting myelomatosis were found Skeletal X rays did not show any abnormalities Less than 2% plasma cells were detected in his bone marrow In Oct 1975 a monoclonal IgG(kappa) paraprotein was detected after agarose gel electrophoresis Two years later he had developed a biclonal gammopathy of IgG(kappa) and IgG(lambda) specificities respectively (44) Biopsy of excised skin tumor material verified the presence of neurofibromas Plasma cells were not detected in the excised tumor material Chromosome analysis demonstrating 46XY karyotype did not reveal any abnormalities His sudden death in Aug 1977 was presumably related to his aorta outflow obstruction No post mortem

*Patient IV 1*

A 29-year-old man with an appearance very similar to his father patient III 1 (Fig 2) Atypical broad face" with the ears "low located broad lips and atypical freckling Excessive freckling and cafe au lait spots on the trunk but few neurofibromas In addition he has an increased kyphoscoliosis of columna and pes equinovarus Like his father he has a rough systolic murmur grade V The diagnosis of aorta outflow obstruction was verified by a cardiologist Electrophoresis of serum on agarose gel demonstrated biclonal gammopathy with IgG(kappa) and IgA(kappa) specificities No skeletal osteolytic lesions were demonstrated Serum albumin was decreased to 25 g/l Chromosomal analysis demonstrated 46XY karyotype without signs of chromosomal instability He was admitted to the hospital because of a leg fracture which is now healed Because of minor mental capacity rehabilitation has been complicated

*Patient IV 2*

Sister of the above discussed patient She also demonstrated excessive freckling and cafe au lait spots indicating von Recklinghausen s disease However she was never diagnosed as having the disease Her mental capacity was said to be low She died in 1962 from a cerebellar tumor a condition which is known to be often associated with Recklinghausen s disease Eventual aortic stenosis or gammopathy were not studied

## METHODS

*Analytical procedures*

Agarose gel electrophoresis of serum proteins was carried out according to Johansson (16) Immunoelectrophoresis for demonstration of gamma alpha and mu heavy chains and kappa and lambda light chains was performed conventionally on glass plates in 1% agarose gel Radial immunodiffusion was adopted from Mancini et al (25)

The size of the monoclonal components was evaluated after determination of total serum protein and specific scanning of individual components in a Gelman automatic scanner with integrator


A


B

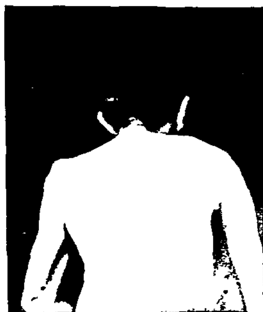

C

Fig 2 Proband IV 1 (A B) Note the increased pigmentation low situated ears and broad extruding lips (C) Classical cafe au lait spots and axillary freckling Note the increased kyphoscoliosis of columna Right sided pes equinovarus was also present (D) Marfan like fingers with tendency to cutis laxa similar to that observed in Ehlers Danlos syndrome


D

Table I *Genetic marker studies of the patients and kindreds involved*

| Subject | Hp | Gc | C6 | Gm a | b | f | g | x |
|---|---|---|---|---|---|---|---|---|
| III 2 | 2 1 | 2 | AB | + | + | + | + | − |
| IV 1 | 2 2 | 2 | A | + | + | + | + | + |
| IV-6 | 2 1 | 2 1s | AB | + | − | − | + | + |
| IV 7 | 2 1 | 2 1f | A | + | + | + | + | − |
| IV 10 | 2 1 | 1f 1s | AB | + | − | − | + | − |
| IV 11 | 2 1 | 2 1s | A | + | + | + | + | + |
| IV 12 | 1 1 | 2 1s | A | + | − | − | + | + |
| V 3 | 1 1 | 1s | AB | + | + | + | + | + |
| V-4 | 1 1 | 1s | AB | + | + | + | + | + |
| V 5 | 2 1 | 2 1s | AB | + | + | + | + | + |
| V-6 | 2 1 | 1f 1s | A | + | + | + | + | − |
| V 7 | 2 2 | 1f 1s | A | + | + | + | + | − |
| V 8 | 2 1 | 1f 1s | AB | + | + | + | + | − |
| V 9 | 2 2 | 1f 1s | AB | + | + | + | + | − |

Light chains in urine were examined after concentration of morning samples 200 times in Amicon filters applying Heller s and Bradshaw s tests for protein In addition electrophoresis and immunoelectrophoresis of concen trated urine were carried out in agarose gel

Films from bone marrow aspirates were fixed in absolute methanol and stained by May Grunwald Giemsa at pH 6 8 for light microscopy

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Blood samples were taken from all available patients their sibs and available parents The samples were analyzed with respect to haptoglobin (Hp) group-specific protein (Gc) and complement factor −6 (C6) serum systems Also genetic markers (Gm) of the constant region of $IgG_1$ and $IgG_3$ heavy chains were tested (antigens a b f g x)

*Isolation and identification of various lymphocyte populations*

These studies were carried out in probands III 1 and IV 1 who both had biclonal gammopathies Lymphocytes were isolated from heparinized whole blood by means of the Isopaque Ficoll gradient centrifugation technique (9) *B lymphocytes* Membrane-bound Ig as determined by the immunofluorescent technique was used as a marker of human B lymphocytes (9) *T lymphocytes* with surface re ceptors for sheep erythrocytes were detected with a roset te technique (9) *EA rosette forming cells* have receptors for the Fc part of the IgG and they were detected after reaction of the lymphocytes with human O R1 R2 eryth rocytes sensitized with anti-(C+D)-IgG antibodies (Ripley) Cytocentrifuge preparations were also made of pe ripheral blood lymphocytes in the conventional way

*Stimulation of lymphocytes with mitogens (patients III 1 and IV 1)*

A sample containing 0 1 ml lymphocytes ($10^6$/ml) was incubated with 1 ml of McCoys 5A medium (Gibco-Biocult Glasgow) with 20% calf serum 0 04 ml phyto-hemagglutinin (PHA) (1 5) (Wellcome Research Labora tories Beckenham) respectively 0 04 ml pokeweed mitogen (PWM) (1 5) (Grand Island Biological Company Grand Island New York) were added to stimulate the all cultures The lymphocytes wer also stimulated with purified protein derivative (PPD) of tuberculin (1 mg/ml) (Sta tens Seruminstitutt Copenhagen) The PHA and PWM stimulated cultures were then incubated at 37°C in 5% $CO_2$ atmosphere for 3 days After 2 days $^3$H thymidine was added, and the number of counts per minute was re gistered after 3 days while PPD-stimulated cultures were terminated after 6 days Controls without PHA PWM and PPD were subjected to the same procedures as well as lymphocytes from normal blood donors All experiments were carried out in triplicate

## *Special Studies of Patient IV 1*

*Identification of T cells bearing receptor for the Fc portion of IgG (Tgamma)*

T-cells were isolated by Isopaque Ficoll gradient centrifugation of lymphocytes which had formed rosettes with sheep red blood cells The erythrocytes in the cell pellets were lyzed with destilled water T lymphocytes with re ceptors for the Fc part of IgG were detected by rosetting them with human O $R_1$ $R_2$ sensitized with anti-(C+D) Ripley antibodies (9)

*Identification of T-cells with receptors for the Fc portion of IgM (Tmu)*

IgM antibodies against ox erythrocytes (ORBC) were obtained as described elsewhere (13) In brief 2% solu tion of ORBC antibodies was sensitized with equal volume of rabbit anti ORBC IgM antibodies in subagglutinin con centration The sensitized ORBC were mixed with equal volumes of isolated T lymphocytes ($3\times10^6$ cells/ml) which had been incubated overnight in medium 199 supple mented with 15% fetal calf serum (FCS) resuspended in phosphate buffered saline supplemented with 0 2% bovine serum albumin After centrifugation at 150 *g* for 5 min at 4°C the mixture was incubated on ice for 60 min The pellet was gently resuspended and the rosette forming cells were counted

*Generation of suppressor cells (patient IV 1 and a normal blood donor)*

Unfractionated lymphocytes $2\times10^6$ were cultured in the presence of optimal mitogenic concentration of concana valin A (Con A) (Sigma Chemical Co St Louis) for 48 hours at 37°C in an atmosphere enriched with 5% car bon dioxide and 100% humidity The control cultures without Con A were also included Cells were treated with mitomycin-C 50 µg/ml for 30 min at 37°C The cells were subsequently washed twice with 0 3 M solution of α meth yl mannoside (14) followed by another three washings in Hanks balanced salt solution The cells were then resus pended at a concentration of $1\times10^6$ cells/ml in RPMI 1640 supplemented with 20% heat inactivated FCS These cells will subsequently be referred to as effector cells

*Suppressor cell assay*

Freshly isolated lymphocytes from one healthy donor were always used as responder cells The suppressor cell assay

Table II *Immunological studies on probands III-1 and IV-1*

All data are within the reference range except for Con A suppression of lymphocytes in patient IV 1 which have no suppressor activity at all

| | III-1 | IV-1 |
|---|---|---|
| Gammopathy | Biclonal | Biclonal |
| M-components | IgG(κ) & IgG(λ) | IgG(κ) & IgA(κ) |
| IgG (g/l) | 27 | 22 |
| IgA (g/l) | 1 1 | Beyond upper limit of quantitation |
| IgM (g/l) | 0 8 | 1 2 |
| B lymphocytes (%) | 11 | 11 |
| T lymphocytes (%) | 71 | 52 |
| Fc receptor-carrying cells (%) | 23 | 24 |
| T lymphocyte reactivity | | |
| PHA response | Normal | Normal |
| PWM response | Normal | Normal |
| PPD response | Normal | Normal |
| Gamma receptor-carrying T cells (%) | Not tested | 13 |
| Mu receptor-carrying T-cells (%) | Not tested | 58 |
| B-lymphocyte reactivity | | |
| PHA response | Normal | Normal |
| PWM response | Normal | Normal |
| Con A suppression (%) | Not tested | −54 |

was performed using microculture plates To each well were added 50 μl of the responder cells (1×10⁶ cells/ml) 50 μl of medium and 50 μl of effector cells (1×10⁶ cells/ml) To each well were added either 20 μl of Con A (75 μg/ml) or 20 μl medium The cells were incubated at 37°C for 72 hours in humidified atmosphere enriched with 5% $CO_2$ The cultures were pulsed 18 hours prior to harvesting with 0 5 mCi of $^3H$ methyl thymidine (Amersham Radiochem ) The cells were harvested on a glass fibre filter plate using a semiautomatic harvesting machine (Skatron A/S Oslo) The radioactivity was counted in a liquid scintillation counter Data were corrected for quenching and expressed as mean disintegration per minute (dpm) and the degree of suppression was calculated according to the following formula

$$\text{Percentage suppression} = 1 - \frac{\text{dpm}_s \text{ ConA}}{\text{dpm}_c \text{ ConA}} \times 100$$

in which $\text{dpm}_c$ Con A is the disintegration per min of the responder cells in presence of control (not stimulated with Con A) and Con A $\text{dpm}_s$ Con A is the disintegration per min of the responder cells in the presence of Con A stimulated cells and Con A

## RESULTS OF LABORATORY STUDIES

*Genetics* The marker studies conducted (Table I) provided no linkage information Chromosomal analysis in probands III 1 and IV 1 demonstrated no chromosomal instability There was no information on inbreeding among the kindreds

*Immunology* Biclonal gammopathy was demonstrated in two subjects III 1 and IV 1 (Table II) No Con A induced suppressor cell activity could be demonstrated in cells from patient IV 1 while normal suppressor cell activity was demonstrated in a presumably healthy blood donor Similar suppression studies were not performed in patient III 1 before his death In these two subjects the relative proportion of B and T lymphocytes and Fc receptor carrying cells were all normal (Table II) Also the B and T lymphocyte reactivity after mitogenic stimulation was normal in the two probands The proportion of gamma and mu receptor carrying T cells were tested in patient IV 1 and found to be normal

*Polyclonal hypergammaglobulinemia* was demonstrated in subjects IV 7 IV 10 V 3 and V 9 without evidence of any underlying disorder including acute phase reactions infections liver diseases collagen diseases or polyarthritis

## DISCUSSION

Neurofibromas are believed to be benign tumors originating from the sheath of Schwann Fibrotic lesions are common in von Recklinghausen's disease having been found in small vessels of the kidney endocrine system gastrointestinal tract and heart (34 35) In addition atypical pigmentation

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Freshly isolated lymphocytes from one healthy donor were *always used as responder cells The suppressor cell assay*

Recent studies have indicated that the expression of humoral immunity by specific antigen stimulated lymphocytes is regulated by helper and suppressor cells (14) These immunoregulatory lymphocytes are T-cells but can possibly also be confined to other cells (14) Thus it has been postulated that abnormalities in the function of suppressor cells may contribute in part to variable hypogamma globulinemia or development of monoclonal compo nents (14) It might therefore be of interest that our patient IV 1 demonstrated no concanavalin generated suppressor cell activity In other words the development of the biclonal components in these patients may possibly in part be conditioned by suppressor T cell deficiency or inadequacy Unfortunately patient III 1 died before the suppressor cell assay was available but it is tempting to assume a similar mechanism in this patient Otherwise the proportion of B and T lymphocytes as well as Fc receptor carrying cells were normal The B and T lymphocyte reactivities were normal after stimulation with PHA PWM and PPD

So far we can conclude that we have observed three cases with von Recklinghausen's disease in the same family of four members an observation which confirms the hypothesis that this disease is probably inherited in an autosomally dominant pattern Two of these patients had in addition aorta outflow obstruction and biclonal gammopathy a syndrome which has previously not been reported Further studies of the other family members showed that aorta stenosis/mors subita occurred frequently but genetic marker studies failed to reveal any linkage between these entities Consequently the syndrome reported is probably restricted to the first kindred studied Detailed biochemical and immunological studies of the monoclonal components are in progress

## REFERENCES


1 Agrawal R L Bhargava S Samma A H Kothari A K Bedi H K & Shrimali R L Empty sella syndrome with neurofibromatosis Indian J Ophthalmol 24 38 1977

2 Barber P V Carcinoid tumour of the ampulla of Vater associated with cutaneous neurofibromatosis Postgrad Med J 52 514 1976

3 Bassan H M & Bitnun S Systemic lupus erythematosus and neurofibromatosis Int J Dermatol 15 36 1976

4 Bednarczyk J & Legiewski A Elephantiasis neuromatosa Chir Narzadow Ruchu Ortop Pol 42 439 1977

5 Cordier J Stehlin B & Bowyer M Optic atrophy and buphthalmia in the course of Recklinghausen's disease Bull Soc Ophtalmol Fr 76 833 1976

6 Duperrat B Dufourmentel C Leis S & Loreneau B Cutis laxa and anethoderma Ann Chir Plast 22 11 1977

7 Fledelius H & Elderup Jørgensen P Optic nerve glioma and phaeochromocytoma associated with von Recklinghausen's disease A case report Br J Ophthalmol 61 240 1977

8 Frykman G K & Wood V E Peripheral nerve hamartoma with macrodactyli in the hand Report of three cases and review of the literature J Hand Surg 3 307 1978

9 Frøland S S & Natvig J B Identification of three *different lymphocyte populations by surface markers* in T and B lymphocytes in humans Transplant Rev 16 114 1973

10 Galanski M & Hoffmann P Multiple vascular stenoses in neurofibromatosis Radiology 17 83 1977

11 Gill P & Singh M Ataxia telangiectasia in siblings J Indian Med Assoc 68 167 1977

12 Gotze P & Kuhne D The socalled Moyamoya syndrome associated with Recklinghausen's neurofibromatosis A case report Nervenarzt 47 34 1971

13 Gupta S & Good R A Subpopulations of human T lymphocytes V T lymphocytes with receptors for immunoglobulin M or G in patients with primary immunodeficiency disorders Clin Immunol Immunopathol 11 292 1978

14 Hodgson H J F Wands J R & Isselbacher K J *Alteration in suppressor cell activity in chronic active* hepatitis Proc Natl Acad Sci USA 75 1549 1978

15 Hoffmann P & Galanski M Congenital bowing of the tibia in neurofibromatosis von Recklinghausen Fortschr Geb Roentgenstr Nuklearmed 125 417 1976

16 Johansson B G Agarose gel electrophoresis Scand J Clin Lab Invest (Suppl) 124 7 1972

17 Kachi T Ando K & Hayashi K Case of von Recklinghausen's disease with recurrent polyneuropathy Jpn J Clin Med 34 3560 1976

18 Kawahara N Kawai H & Maruyama Y Von Recklinghausen's disease with a malignant change and association with giant stomach neoplasm Nippon Rinsho 35 2662 1977

19 Keller R T & Logan G M Jr Adenocarcinoma of the pancreas associated with neurofibromatosis Cancer 39 1264 1977

20 Kitao T Miyabo S & Hattori K Hemophilia associated with von Recklinghausen's disease South Med J 69 16 1976

21 Kumar S Gulati D R & Mann K S Multiple astrocytoma of spinal cord in von Recklinghausen's disease A case report Neurol India 25 247 1977

22 Kyle R A & Bayrd E D The monoclonal gammopathies Amer Lecture Series pp 154–55 Thomas Springfield Ill 1976

23 Lacombe M A Gynecomastia in neurofibromatosis Report of a case J Maine Med Assoc 67 334 1976

24 Macaulay R A Neurofibrosarcoma of the radial

nerve in von Recklinghausen s disease with metastatic angiosarcoma J Neurol Neurosurg Psychiatry 41 474 1978

25 Mancini G Vaerman, J P Carbonara A O & Heremans J F A single radial diffusion method for the immunological quantitation of proteins Prot Biol Fluids 11 370 1963

26 Morishima T Nagashima N Ishikawa T, Koshinaga K & Endo M Melanin producing cells in neurofibromatous lesion of Recklinghausen s disease Jpn J Dermatol 86 197 1976

27 Payeur M G Rey Goetz M L Beauvais P Sussmann D & Barthelme B A case of congenital von Recklinghausen s disease with glaucoma Bull Soc Ophtalmol Fr 76 177 1976

28 Per Lee J H & Clairmont A A Acoustic neurinoma in von Recklinghausen s disease South Med J 69 844 1976

29 Pless J Roed Petersen K & Nielsen K Macroglossia neurofibromatosa Ugeskr Laeger 139 655 1977

30 Poleksic S Leiomyosarcoma of urinary bladder in von Recklinghausen s neurofibromatosis Urology 10 341 1977

31 Prager P J Frank P Blassmann K H & Betz H Congenital skull changes in Recklinghausen s neurofibromatosis Strahlenterpie 152 43 1976

32 Raskin J B & Dodd H Juvenile polyposis coli concurrent with neurofibromatosis South Med J 69 1374 1976

33 Reich S D & Wiernick P H Von Recklinghausen s neurofibromatosis and acute leukemia Am J Dis Child 130 888 1976

34 Reubi F Les vaisseaux et les glandes endocrines dans la neurofibromatose le syndrome sympathicotonique dans la maladie de Recklinghausen Schweiz Z Path 7 168 1944

35 — Neurofibromatose et lesions vasculaires Schweiz Med Wochenschr 75 463 1945

36 Rosenbusch G Hoefinagels W H Koene R A Penn W & Thijssen H O Renovascular hypertension in neurofibromatosis Simultaneous occurrence of multiple abdominal and cerebral vascular abnormalities Fortschr Geb Roentgenstr Nuklearmed 126 218 1977

37 Rosenquist G C Krovetz L J Haller J A Simon L A & Bannayan G A Acquired right ventricular outflow obstruction in a child with neurofibromatosis Am Heart J 79 103 1970

38 Soll D B Anophthalmos and neurofibromatosis In Techniques of anophthalmic cosmesis (ed P Guibor and M Guibor) p 137 Stratton New York 1976

39 Stay E J & Vawter G The relationship between nephroblastoma and neurofibromatosis Cancer 39 2550 1977

40 Toman J Mandibular deformation due to benign tumours Riv Ital Stomatol 46 38 1977

41 Turek M Raistrick E R & Hart C D Retinal tumours in neurofibromatosis Can J Ophthalmol 12 68 1970

42 Walden P A Johnson A G & Bagshawe K D Wilms tumour and neurofibromatosis Br Med J 1 (6064) 813 1977

43 Webb W R & Goodman P C Fibrosing alveolitis in patients with neurofibromatosis Radiology 122 289 1977

44 Wille L E & Østborg J Development of biclonal gammopathy in a patient with von Recklinghausen s neurofibromatosis Acta Med Scand 205 243 1979

Acta Med Scand 207 305–307 1980

# Failure of Excessive Doses of Ampicillin to Prevent Bacterial Relapse in the Treatment of Acute Pyelonephritis

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*From the Departments of Infectious Diseases and Clinical Bacteriology University of Lund Malmo General Hospital Malmo Sweden*

ABSTRACT In order to evaluate whether very high doses of ampicillin might be more effective than conventional therapy in eradicating bacteria in patients with acute pyelonephritis, 34 affected patients were randomly assigned into two treatment groups One group was given ampicillin in a daily dose of 30 g for three days and 20 g for four days without further treatment The other group was given ampicillin in moderate doses for one month Out of 13 patients treated with excessive doses for one week, only three were completely cured whereas conventional therapy cured 9 out of 21 Thus, excessive doses of ampicillin given for one week were not more effective but more expensive and possibly less beneficial than conventional therapy

*Key words* ampicillin pyelonephritis

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The minimal duration of treatment necessary to eradicate bacteria in urinary tract infection is debated at present A single oral dose of sulphafurazole has been found to eradicate bacteria in children with lower urinary tract infection (2) Ronald et al (3) reported that 36 of 39 women with infection confined to the bladder were cured with a single i m injection of kanamycin whereas 47 of 65 patients with infection originating from the upper urinary tract relapsed almost immediately Fang et al (1) found a single 3 gram oral dose of amoxicillin effective in all their patients with bladder infection whereas a dose of 250 mg four times a day for ten days was effective in only 9 out of 18 patients with renal infection

In the treatment of severe infections in small children the pediatricians successfully use ampicillin in a daily dose of 400 mg/kg b wt Such large doses have rarely been administered to adults

The aim of the present controlled investigation was to assess the effect of short term treatment of acute pyelonephritis in adults with ampicillin in a daily dose of 400 mg/kg b wt

## PATIENTS AND METHODS

Among 75 patients with a preliminary diagnosis of acute pyelonephritis urine cultures corroborated the diagnosis in 42 In eight patients the effect of treatment could not be assessed because of premature discontinuation of treatment or follow up The remaining 34 patients met the criteria for participation and were included in the study They were randomly assigned into two groups treated with large or moderate doses of ampicillin Patients with indwelling urinary catheters did not participate
dwelling urinary catheters did not participate

Group 1 was given ampicillin in an i v dose of 10 g every 8 hours on days 1–3 and 10 g every 12 hours on days 4–7 No further treatment was given Group 2 was given ampicillin in an i v dose of 2 g every 6 hours on days 1–3 On days 4–7 treatment was continued either as before or with pivampicillin orally 0 35 g three times a day Thereafter oral therapy with pivampicillin in the same dose was continued for five weeks The ampicillin preparations used were Pentrexyl® (Bristol) or Doktacillin® (Astra) and the pivampicillin preparation was Pondocillin® (Leo)

The patients were checked up at 3 5 10 14 and 27 weeks after initiation of treatment A yield of more than $10^5$ bacteria per ml urine in two samples or in one sample together with a positive nitrite test was considered a sign of relapse or reinfection All cultures were carried out by conventional methods at the Institute of Clinical Bacteriology Serum levels of ampicillin were occasionally measured on day 3 using an agar well method

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Table I *Clinical data on the patients and outcome of ampicillin treatment*
RS = renal stones PN = papillary necrosis NG = no growth

| Case no | Age (y) | Sex | Initial daily dose (g) | Duration of treatment (weeks) | Initial temperature (C) | Pyuria in urinary sediment (arbitrary) scale) | Leucocytes ($10^9$/l) | ESR (mm in 1st h) | S-creatinine (µmol/l) | Predisposing factors |
|---|---|---|---|---|---|---|---|---|---|---|
| *Group 1* | | | | | | | | | | |
| 1 | 43 | ♀ | 30 | 1 | 38 6 | ++ | 7 3 | 21 | 100 | |
| 2 | 53 | ♂ | 30 | 1 | 38 9 | +++ | 7 3 | 65 | 80 | |
| 3 | 59 | ♀ | 30 | 1 | 40 0 | +++ | 11 7 | 46 | 70 | |
| 4 | 50 | ♀ | 30 | 1 | 38 0 | +++ | 8 0 | 55 | | |
| 5 | 84 | ♀ | 30 | 1 | 39 0 | +++ | 10 8 | 65 | 160 | |
| 6 | 16 | ♀ | 30 | 1 | 37 8 | +++ | 9 5 | 26 | 60 | |
| 7 | 65 | ♀ | 30 | 1 | 39 7 | ++ | 7 1 | 78 | 100 | |
| 8 | 73 | ♀ | 30 | 1 | 40 0 | +++ | 10 8 | 108 | 100 | |
| 9 | 68 | ♀ | 30 | 1 | 39 9 | +++ | 29 3 | 50 | 90 | |
| 10 | 26 | ♀ | 30 | 1 | 39 6 | +++ | 11 3 | 20 | 70 | |
| 11 | 17 | ♀ | 30 | 1 | 39 4 | +++ | 12 1 | 122 | 80 | |
| 12 | 62 | ♀ | 30 | 1 | 40 0 | +++ | 8 0 | 73 | 110 | |
| 13 | 60 | ♀ | 30 | 1 | 39 0 | +++ | 5 9 | 62 | 100 | |
| *Group 2* | | | | | | | | | | |
| 14 | 71 | ♀ | 8 | 6 | 40 4 | +++ | 10 1 | 63 | 100 | RS |
| 15 | 62 | ♀ | 8 | 6 | 39 0 | +++ | 6 1 | 112 | 120 | |
| 16 | 20 | ♀ | 8 | 6 | 39 7 | +++ | 27 4 | 155 | 120 | |
| 17 | 44 | ♀ | 8 | 6 | 39 4 | ++ | 11 7 | 19 | 90 | RS |
| 18 | 21 | ♀ | 8 | 6 | 39 0 | ++ | 13 7 | 71 | 90 | |
| 19 | 73 | ♀ | 8 | 6 | 39 3 | ++ | 8 0 | 7 | 90 | |
| 20 | 62 | ♀ | 8 | 6 | 39 2 | +++ | 8 4 | 33 | 80 | RS |
| 21 | 50 | ♀ | 8 | 6 | 39 9 | +++ | 13 8 | 21 | 120 | |
| 22 | 50 | ♀ | 8 | 6 | 39 5 | +++ | 15 3 | 42 | 70 | |
| 23 | 21 | ♀ | 8 | 6 | 40 1 | +++ | 9 7 | 63 | 90 | |
| 24 | 64 | ♀ | 8 | 6 | 37 4 | +++ | 16.5 | 6 | 90 | PN |
| 25 | 63 | ♂ | 8 | 6 | 39 9 | +++ | 4 5 | 8 | 120 | |
| 26 | 23 | ♀ | 8 | 6 | 40 0 | +++ | 7 2 | 38 | 60 | |
| 27 | 18 | ♀ | 8 | 6 | 40 0 | +++ | 10 1 | 46 | 60 | |
| 28 | 54 | ♀ | 8 | 6 | 39 0 | ++ | 15 7 | 48 | 80 | |
| 29 | 33 | ♀ | 8 | 6 | 39 0 | +++ | 8 7 | 68 | 70 | |
| 30 | 45 | ♀ | 8 | 6 | 39 6 | +++ | 6 6 | 47 | 40 | |
| 31 | 51 | ♀ | 8 | 6 | 39 6 | +++ | 6 4 | 41 | 90 | |
| 32 | 50 | ♀ | 8 | 6 | 40 5 | ++ | 8 8 | 55 | 100 | |
| 33 | 27 | ♂ | 8 | 6 | 40 2 | ++ | 16 8 | 24 | 110 | |
| 34 | 69 | ♂ | 8 | 6 | 38 8 | +++ | 9 5 | 71 | 120 | |

## RESULTS

An i v injection of 10 g of ampicillin on the third day was followed by peak concentrations up to 460 mg/l and trough values after eight hours of 9 mg/l

Clinically the response to treatment was excellent in all except two patients in group 2 In the others body temperature returned to normal within a few days Exanthema occurred occasionally and one patient who had been treated with large doses of ampicillin showed transient renal failure

The bacteriological response was less rewarding (Table I) Treatment turned out to be successful in three of the 13 patients treated with large doses of ampicillin for one week seven relapsed and three were reinfected Among the 21 patients treated with moderate doses for a longer time the outcome was successful in nine six relapsed and six were reinfected

Thus excessive doses of ampicillin administered during one week were not superior to the less expensive conventional therapy

| Spec es | | S de-effects | Time after start of treatment at which bacteria reappeared | Evaluation |
|---|---|---|---|---|
| Before treatment | At follow-up | | | |
| E coli | E col | | 3 weeks | Relapse |
| E coli | E coli | | 3 weeks | Relapse |
| E coli | E col | Exanthema | 3 weeks | Relapse |
| E col | E coli | Exanthema | 3 weeks | Relapse |
| *E coli* | *E coli resistant* | | *3 weeks* | *Re nfection* |
| E coli | E coli | Exanthema | 3 weeks | Relapse |
| E col | E coli | Renal dysfunction | 3 weeks | Relapse |
| E coli | E col | | 3 weeks | Relapse |
| F coli | Klebs ella | | 2 months | Reinfect on |
| E col | E coli | | 4 months | Reinfection |
| E coli | NG | | | Su cessful |
| E coli | NG | | | Successful |
| E col | NG | | | Successful |
| E coli | E coli | | 1 week | Relapse |
| E coli | Klebs ella | | ? weeks | Reinfection |
| E coli | E coli res stant | | 3 weeks | Reinfection |
| E coli | E coli | | 3 weeks | Relapse |
| Streptococci | E coli | | 3 weeks | Reinfection |
| E coli | E coli | | 6 months | Reinfect on |
| E coli | E coli | | 2 months | Relapse |
| E col | F coli | | ? months | Relapse |
| E coli | E col | | 2 months | Relapse |
| E coli | P mirab l s | | 2 months | Reinfect on |
| E coli | Enterococci | | 2 months | Reinfection |
| E col | E coli | | ? months | Relapse |
| E coli | NG | | | Successful |
| E coli | NG | | | Successful |
| E coli | NG | Exanthema | | Successful |
| E col | NG | | | Successful |
| E coli | NG | | | Successful |
| E coli | NG | | | Successful |
| E col | NG | | | Succes ful |
| E col | NG | | | Successful |
| E coli | NG | | | Successful |

## COMMENT

Persistent bacteriuria after an episode of acute pyelonephritis may endanger future renal function The failure of excessive doses of amp c ll n to rel ably erad cate the infect on shows that other ways *must be devised for complete cure of the d sease*

## REFERENCES

1 Fang L S T Tolkoff Rubin N E & Rubin R H Eff acy of s ngle-dose and convent onal amoxic ll n *therapy n urinary tract infection localized by the anti* body-coated bacteria techn c N Engl J Med ?98 413 19?8

? Kallen us G & W nberg J *Urinary tract infection* treated w th single dose of short acting sulphonamide Br Med J 1 1175 19?9

3 Ronald A R Boutros P & Mourtada H Bacteriuria localization and response to single-dose therapy in women JAMA ?35 1854 19?6.

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# Renal Concentrating Capacity in Long-Term Lithium Treatment and after Withdrawal of Lithium

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ABSTRACT The urinary concentrating capacity was estimated with the DDAVP test in 87 patients receiving lithium therapy, which was discontinued in all patients The test was repeated three and eight weeks after withdrawal of lithium in 75 patients and one year after withdrawal in 27 patients Of the 87 patients 52 were also treated with neuroleptics, which treatment was continued throughout the study Two control groups, consisting of 30 patients receiving only neuroleptics and 30 healthy subjects were studied on one occasion with the DDAVP test Lithium treated patients had significantly lower concentrating capacity and higher serum creatinine than healthy subjects at all examinations Small but statistically significant correlations were found between urinary osmolality and total dose of lithium, between urinary osmolality and duration of lithium treatment, between the highest serum lithium concentration recorded and urinary osmolality after withdrawal of lithium and between the daily dose of lithium and urinary osmolality, while patients were still on lithium The concentrating capacity improved significantly during the first two months after withdrawal of lithium, but not later One year after withdrawal of lithium, 17 of 27 patients still had a concentrating capacity below 800 mOsm/kg Patients receiving lithium and neuroleptics had lower concentrating capacity than patients treated with lithium alone and patients treated with neuroleptics alone had lower concentrating capacity than healthy subjects

*Key words* renal concentrating capacity lithium treatment diabetes insipidus

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Hanlon et al (15) reported in 1949 on the use of lithium salts as a substitute for sodium salt in cardiac decompensation but the results have been described as tragic since some patients died from lithium poisoning (11) Cade (9) reported that lithium had a tranquilizing effect on hyperactive patients and it was thereafter established that lithium is effective in the treatment of manic depressive psychosis (25 26) Lithium salts may now be considered standard drugs in the treatment of manic-depressive psychoses but are also used to a certain extent in other mental conditions

Polyuria polydipsia is a well known side effect of lithium treatment (2 5 14 17 27) Forrest et al (14) showed that polyuria polydipsia in lithium treated patients was unresponsive to vasopressin Lithium was found to have a toxic effect on dog kidney as early as in 1950 (23) Light microscopy showed lesions with a chronic appearance mainly in the distal tubules and collecting ducts Later studies have confirmed the occurrence of similar lesions in lithium treated rats (12 24) even in small doses (13) Occurrence of renal lesions in human lithium intoxication has been known for several years Chapman and Lewis (10) described morphologic changes in a case of lithium intoxication The lesions found in that case and in a case of lithium induced diabetes insipidus were similar to those found in animals (18) Hestbech et al (16) described renal lesions in 14 patients who were examined because of lithium intoxication or polyuria All of them showed impairment of the renal concentrating capacity Renal biospies showed focal nephron atrophy and interstitial fibrosis Recently Burrows et al (8) found a kind of tubular lesion which they considered to be specific for lithium treatment

Our aim was to study the frequency of impaired kidney function due to lithium treatment Since earlier investigators have found an impairment of the renal concentrating capacity during lithium therapy but have considered as a rule that this effect is functional and reversible we chose to perform our study after cessation of lithium therapy This study was started in 1977 when the findings of Hestbech et al (16) became known

Table I *Characteristics of patients and healthy subjects (mean ± S D )*

| | Group A (lithium alone) | Group B (lithium plus neuroleptics) | Groups A + B | Group C (neuroleptics alone) | Group D (healthy subjects) |
|---|---|---|---|---|---|
| No of subj | 35 | 52 | 87 | 30 | 30 |
| Age (y ) | 48±12 | 44±12 | 46±12 | 39±12 | 39±9 |
| Previous lithium intoxication | 1 | 5 | 6 | | |
| Hypothyroidism | 4 | 6 | 10 | | |
| Duration of lithium treatment (mo ) | 83±35 | 63±44 | 71±41 | | |
| Total dose of lithium (g) | 454±181 | 434±320 | 442±272 | | |
| *Serum lithium (mmol/l)** | | | | | |
| Mean | 0 7±0 1 | 0 8±0 1 | 0 8±0 1 | | |
| Maximum | 1 1±0 2 | 1 3±0 3 | 1 2±0 3 | | |

* During previous lithium therapy

## STUDY POPULATION AND METHODS

Persons over 65 years of age or with diabetes mellitus drug abuse or known renal and urinary tract disease were excluded from the study With these exceptions we examined all lithium treated in patients at Umedalen Hospital and the Department of Psychiatry University of Umeå During a 3-month period we also examined all lithium treated out patients coming for regular check up Another 20 patients on lithium for more than five years were requested to come for examination The following groups were examined

*Lithium treated patients*

Eighty seven patients (41 males and 46 females) were examined Their mean age was 46±12 years (Table I) They were separated into two groups Group A ($n$=35) *received lithium therapy only* and *group B* ($n$=52) was treated with lithium plus neuroleptic drugs (phenothiazines phenothiazine derivatives and butyrophenones)

From the patient journals we calculated how many months the patients had received lithium therapy and the total dose of lithium consumed (Table I) Occurrence of previous lithium intoxication was noted (Table I) Ten patients received substitution therapy for hypothyroidism One patient was found with unsubstituted hypothyroidism We also extracted from the journals all previous determinations of serum lithium concentration which had been performed every third month or more often in blood samples taken 12 hours after the last intake of lithium *The daily dose of lithium had always been divided* The mean serum lithium concentration was calculated and the highest single value found in each individual was noted (Table I)

The last lithium dose was administered 12 hours before examination I when the serum concentrations of electrolytes creatinine and lithium were determined DDAVP 40 µg (Minirin* Ferring Malmö Sweden) was administered intranasally and the patients were requested to empty the bladder 30–60 min thereafter They left urine samples 3–4 hours later for determination of osmolality Protein and glucose were determined qualitatively

The examination was repeated three (examination II) and eight weeks (examination *III*) after withdrawal of lithium There was however a drop-out of 12 patients Lithium therapy in 11 of these cases had been reinstituted before examination III because of suspected or demonstrated mental deterioration One patient refused further cooperation after examination I Some patients ($n$=27) living at or near the hospital were also examined 12 months after cessation of lithium therapy (examination IV) The number of patients in whom lithium treatment was continued after examination III was not estimated

*Patients on neuroleptic drugs alone and healthy subjects*

Since many of the lithium treated patients were also treated with neuroleptics we decided to examine 30 patients who were treated with neuroleptics alone (group C) All 30 (19 males 11 females) were in patients and most of them had schizophrenia (Table I) For comparison 30 healthy volunteers (group D 11 males 19 females) were also examined (Table I) They were members of the hospital staff doctors and nurses who were requested to participate Like the patients they were asked about urinary tract symptoms Subjects with such symptoms now or earlier were not included The subjects in groups C and D underwent the DDAVP test and other laboratory examinations as described above but only once

*Statistics*

Mean values standard deviations Student s $t$ test $t$ test of pairs and Pearson s correlation coefficient were used A level of significance of $p \leq 0\ 05$ was chosen

## RESULTS

*All lithium treated patients* (groups A + B)

Only three out of 84 patients had a normal concentrating capacity (≥800 mOsm/kg $H_2O$) during lithium therapy (Table II) Three weeks after cessation of lithium therapy the mean urinary concentrating capacity had increased from 517±197 to 605±202 mOsm The osmolality eight weeks after withdrawal of lithium was 658±181 mOsm (Table II) The osmolality increment between the examinations was statistically significant ($t$ test $p$=0 001)

Table II *Urinary osmolality (DDAVP test) and serum creatinine in patients 0 (I) three (II) and eight (III) weeks after cessation of lithium treatment and in healthy subjects (mean ± S D )*

| Group | Urinary osmolality (mOsm/kg $H_2O$) | | | Serum creatinine (μmol/l) | | |
|---|---|---|---|---|---|---|
| | I | II | III | I | II | III |
| A | 577+197 (*n* 33) | 676±147 (*n*=35) | 708+156 (*n* 32) | 81+13 (*n* 33) | 76+14 (*n* 35) | 81+[illegible]0 (*n* 30) |
| B | 478+ 09 (*n* 51) | 556+222 (*n*=51) | 6[illegible]1+192 (*n* 43) | 82+18 (*n* 52) | 79+15 (*n* 51) | 75±13 (*n* 42) |
| A+B | 517+197 (*n* 84) | 605+[illegible]03 (*n*=86) | 658+182 (*n* 75) | 82+16 (*n* 83) | 78+15 (*n* 86) | 78±16 (*n* 7[illegible]) |
| C | | 834±136 (*n* 30) | | | 81+24 (*n*=[illegible]4) | |
| D | | 901+101 (*n* 30) | | | 0+14 (*n* [illegible]9) | |
| Statistics (Student's *t* test *p*≤) | | | | | | |
| A vs B | 0 024 | 0 003 | 0 040 | n s | n s | n s |
| A vs C | 0 001 | 0 001 | 0 001 | n s | n s | n s |
| A vs D | 0 001 | 0 001 | 0 001 | 0 002 | n s | 0 014 |
| B vs C | 0 001 | 0 001 | 0 001 | n s | n s | n s |
| B vs D | 0 001 | 0 001 | 0 001 | 0 001 | 0 008 | n s |
| A+B vs C | 0 001 | 0 001 | 0 001 | n s | n s | n s |
| A+B vs D | 0 001 | 0 001 | 0 001 | 0 001 | 0 01[illegible] | 0 022 |
| C vs D | | 0 034 | | | 0 037 | |

but the concentrating capacity eight weeks after withdrawal of lithium was still significantly lower than in healthy subjects (Fig 1)

The total dose of lithium consumed by each patient was compared to the urinary osmolality at examinations I II and III (Fig 1) and IV The osmolality was found to decrease with increasing total dose of lithium (The correlation coefficients were I r= 0 21 p 0 028 II r 0 34 p−0 001 III r− 0 28 p 0 008 IV r− 0 31 p 0 107 )

Twenty seven patients 13 males and 14 females with a mean age of 44 years were examined one year after discontinuation of lithium therapy The total dose of lithium administered to each of these

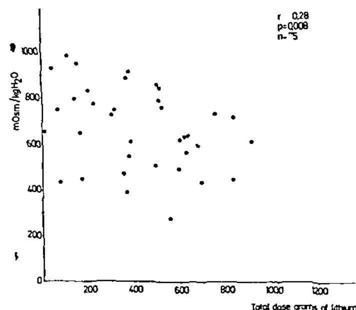



*Fig 1* Urinary concentrating capacity eight weeks after cessation of lithium therapy in relation to total dose of lithium

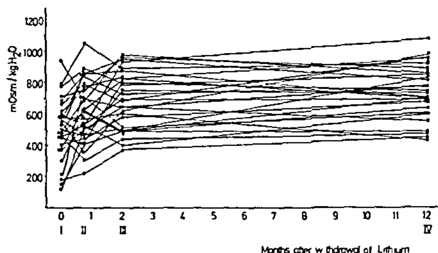



*Fig 2* Urinary concentrating capacity during lithium therapy and after withdrawal of lithium in 27 patients $t$ test of pairs did not yield a significant difference ($p$=0 096) between examinations III and IV

patients was 380±318 g/l—a smaller dose than that given to the main group of patients studied for two months only The mean osmolality values of the 27 patients followed for one year and of the total patient population were (in that order) I 512 517 II 611 605 III 683 658 IV 733 The osmolality values found at examination IV 12 months after lithium therapy did not differ significantly from those found in the main group at examinations I II and III Fig 2 shows that the concentrating capacity did not increase significantly after two months ($t$ test of pairs $p$=0 096 )

The duration of lithium treatment (mean 71 months) was compared to the concentrating capacity The osmolality was found to decrease with increasing duration of lithium treatment (III $r$=−0 37 $p$=0 001)

A correlation was also found between the highest serum lithium value during previous lithium therapy and urinary osmolality eight weeks after withdrawal of lithium ($r$=−0 32 $p$=0 003) (Fig 3) The possible influence of high serum lithium concentration was also studied in a similar way six patients who had a previous history of lithium intoxication and all other patients with a previous maximum serum lithium concentration of 1 2 mmol/l or more formed one group ($n$=27) which was compared to patients with previous maximum serum lithium levels of less than 1 2 mmol/l ($n$=48) The urinary osmolality eight weeks after withdrawal of lithium was 582±171 mOsm in the former group and 701±175 in the latter The difference was significant ($p$=0 006)

The concentrating capacity during lithium therapy (examination I) was found to decrease with increasing daily dose of lithium ($r$=−0 32 $p$ 0 016) Such a correlation between daily do and concentrating capacity was not found at lat examinations A significant correlation was four between serum lithium concentration at examin tion I and urinary osmolality I The mean seru lithium concentration during previous therapy d not correlate significantly with osmolality value No significant correlation was found between dur tion of lithium treatment and maximum seru lithium concentration

The serum creatinine concentration was signif cantly higher in lithium treated patients than i healthy subjects (Table II) There was a significar correlation ($r$=0 33 $p$=0 001) between serum cr atinine concentration two months after cessatic of lithium and duration of the therapy $T$ test c pairs did not yield any significant difference be tween creatinine concentrations at examinations

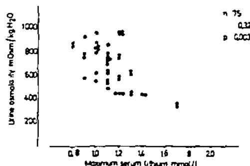



*Fig 3* Urinary concentrating capacity eight weeks afte cessation of lithium therapy in relation to maximum serum lithium during previous lithium therapy

II and III Serum sodium and serum potassium concentrations were normal at all examinations Patients with substituted hypothyroidism did not differ from the others with respect to osmolality and creatinine values Mild proteinuria was found in two instances glucosuria in one

*Patients treated with neuroleptic drugs* (groups B + C)

Patients treated with the combination therapy (group B) had significantly lower concentrating capacity than patients treated with lithium alone (group A) (Table II) Similarly patients treated with neuroleptics alone (group C) had significantly lower concentrating capacity than healthy subjects (group D) (Table II) Patients on neuroleptics alone also had higher serum creatinine concentrations than healthy subjects (Table II) Patients treated with lithium plus neuroleptics had significantly higher mean serum creatinine concentration than healthy subjects at the first two examinations (Table II)

## DISCUSSION

With the exceptions previously stated we examined all lithium treated patients living at or near the hospital who were judged able to cooperate We have no reason to believe that our patient series was selected in any other way Some of our patients were not judged capable of performing an ordinary thirst dehydration test both for psychiatric reasons and because of excessive thirst The DDAVP test was chosen because of its simplicity and the possibility of controlling the patients during the test It has been shown that the DDAVP test yields results comparable to 18–22 hours of fluid deprivation (3 20) Lithium has however been found to suppress the action of the antidiuretic hormone in the kidney tubule (5 19 22) This effect of lithium might induce error in the DDAVP test but we have obtained similar results with DDAVP test and fluid deprivation during lithium therapy (4)

Our results indicate that long term lithium therapy may lead to impairment of the concentrating capacity in most patients The concentrating capacity improved during the first two months after cessation of lithium therapy but remained lower than in healthy subjects even one year later Of 27 patients examined one year after withdrawal of lithium 17 had a concentrating capacity of less than 800 mOsm/kg These 27 patients were representative for our total patient population The finding of a correlation between the total dose of lithium and the duration of lithium treatment on the one hand and the concentrating capacity two months after withdrawal of lithium on the other shows in our opinion that the impairment of the concentrating capacity can be attributed to lithium therapy Moreover the serum creatinine concentration was higher in our previously lithium treated patients than in healthy subjects These findings could be explained by a structural damage to the nephron by lithium Lithium however also has an immediate effect on the concentrating capacity which explains the correlation between daily dose of lithium and urine osmolality at examination I This effect seems to be reversible explaining the improvement after withdrawal of lithium The nephrotoxicity of lithium does not seem to be restricted to serum lithium levels generally known to be toxic since only 11 of our patients had had high serum lithium concentrations (1 5 mmol/l or more) Nevertheless we found a correlation between maximum serum lithium concentration and impairment of renal concentrating capacity One explanation of this finding could be that increasing duration of treatment would also increase the chance of finding a high serum lithium value but we did not find any correlation between duration of lithium therapy and maximum serum lithium concentration The possibility that toxic lithium levels might be the main cause of impairment of concentrating capacity is however not excluded

In this study we also found evidence of an influence of neuroleptic drugs upon kidney function This was demonstrated both in patients treated with neuroleptics plus lithium and in patients treated with neuroleptics alone Patients receiving combination therapy had lower concentrating capacity than those exposed to lithium alone Patients taking neuroleptics alone had lower concentrating capacity and higher serum creatinine than healthy volunteers It should be remembered however that neuroleptic therapy was not discontinued so we do not know whether this effect is reversible or not Another reason for a cautious interpretation of these results is that most patients taking neuroleptics were hospitalized whereas the healthy volunteers were not Similarly patients receiving the combination therapy were hospitalized to a greater extent than patients treated with lithium alone Hospitalized persons have been found to h-

concentrating capacity than healthy volunteers (21) but as far as we know not a higher serum creatinine Patients treated with lithium plus neuroleptics had also had higher daily doses of lithium than patients treated with lithium alone

It might be concluded that we have confirmed previous investigators finding of a reduced concentrating capacity in lithium treated patients Two different mechanisms may be involved one dose-dependent and reversible probably an effect of suppression by lithium of the antidiuretic hormone activity in the kidney the other mechanism does not seem to be dependent upon the daily dose but related to the duration of lithium treatment total dose of lithium and high lithium concentrations This *second effect might be the result of a structural* damage to the nephron It seems to be permanent We cannot yet assess the consequences of this supposed renal damage Our results indicate that there might be a risk of continuously deteriorating renal function with lithium treatment It is surprising that some patients have been on lithium for several years without any sign of impairment of renal function while others seem to develop a deteriorated concentrating capacity relatively fast This difference in the renal response to lithium can possibly be explained by the fact that we cannot ascertain that an individual patient really takes his medicine Anyway it must be important to use lithium only on strict indications with careful control of serum lithium concentration (1) It is possible that the therapeutic levels in general use are unnecessarily high We also consider it important to be aware of the occurrence of severe polyuria during lithium treatment because polyuria per se may be a risk factor (6 7)

## REFERENCES


1 Amdisen A Serum levels monitoring and clinical pharmacokinetics of lithium Clin Pharmacokinet 2 73 1977
2 Angrist B M Gershon S G Levitan S J et al Lithium induced diabetes insipidus like syndrome Compr Psychiatry 11 141 1970
3 Aronson A S & Svenningsen N W DDAVP test for estimation of renal concentrating capacity in infants and children Arch Dis Child 49 654 1974
4 Asplund K Wahlin A & Rapp W DDAVP test in assessment of renal function during lithium therapy Lancet 1 491 1979
5 Baylis P H & Heath D A Water disturbances in patients treated with oral lithium carbonate Ann Intern Med 88 607 1978
6 Bucht G & Wahlin A Impairment of renal concentrating capacity by lithium Lancet 2 778 1978
7 — Impairment of renal concentrating capacity by lithium Lancet 2 580 1978
8 Burrows G D Davies B & Kincaid Smith P Unique tubular lesions after lithium Lancet 1 1310 1978
9 Cade J F J Lithium salts in the treatment of psychotic excitement Med J Aust 2 349 1949
10 Chapman A J & Lewis G Iatrogenic lithium poisoning A case report with necropsy findings J Okla State Med Assoc 65 591 1972
11 Corcoran A C Taylor R D & Page I H Lithium poisoning from use of salt substitutes JAMA 139 685 1949
12 Evan A P The effect of lithium carbonate on the rat kidney an ultrastructural functional and biochemical study Doctoral dissertation University of North Dakota 1971
13 Evan A P & Ollerich D A The effect of lithium carbonate on the structure of the rat kidney Am J Anat 134 97 1972
14 Forrest J N Cohen A D Torretti J et al On the mechanism of lithium induced diabetes insipidus in man and the rat J Clin Invest 53 1115 1974
15 Hanlon L W Romaine M Gilroy F J et al Lithium chloride as a substitute for sodium chloride in the diet JAMA 139 688 1949
16 Hestbech J Hansen H E Amdisen A et al Chronic renal lesions following long term treatment with lithium Kidney Int 12 205 1977
17 Lee R V Jampol L M & Brown W G Nephrogenic diabetes insipidus and lithium intoxication—complications of lithium carbonate therapy N Engl J Med 284 93 1971
18 Lindop G B M & Padfield P L The renal pathology in a case of lithium induced diabetes insipidus J Clin Pathol 28 472 1975
19 Mac Neil S Jennings G Eastwood P R et al Lithium and the antidiuretic hormone Br J Clin Pharmacol 3 305 1976
20 Monn E & Åbyholm G Intranasal DDAVP test in children with chronic pyelonephritis/hydronephrosis 4th International Symposium of Pediatric Nephrology Helsinki 1–4 Aug 1977
21 Monson J P & Richards P Desmopressin urine concentration test Br Med J 1 24 1978
22 Padfield P L Park S J Morton J J et al Plasma levels of antidiuretic hormone in patients receiving prolonged lithium therapy Br J Psychiatry 130 144 1977
23 Radomski J L Fuyat H N Nelson A A et al The toxic effects excretion and distribution of lithium chloride J Pharm 100 429 1950
24 Schou M Lithium studies 1 Toxicity Acta Pharmacol Toxicol 15 70 1958
25 — Lithium in psychiatric therapy Psychopharmacologia 1 65 1959
26 Schou M Juel Nielsen N Strömgren E et al The treatment of manic psychosis by administration of lithium salts J Neurol Psychiatry 17 250 1954
27 Singer I Rotenberg D & Puschett J B Lithium induced nephrogenic diabetes insipidus In vivo and in vitro studies J Clin Invest 51 1081 1972

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# Azathioprine and Subacute Myelomonocytic Leukemia

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**ABSTRACT** The occurrence of subacute myelomonocytic leukemia is reported in a patient who had been treated with azathioprine for six years because of systemic lupus erythematosus. A review of the literature yielded 16 additional patients with non lymphocytic leukemia after the use of azathioprine.

*Key words* leukemia azathioprine systemic lupus erythematosus immunosuppression

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Exposure of human populations to various forms of radiation is followed by an increased incidence of leukemia (15). In the last 15 years certain drug therapies have been associated with a similar effect not only in the case of malignant but also non malignant diseases. Several reports have appeared on the development of second malignancies after treatment for malignant diseases especially Hodgkin s disease (5) and multiple myeloma (12 17 27). It seems that alkylating agents in particular play an important role (17).

There is also an increasing number of reports on the development of malignancies during or after treatment of a non malignant disease. Immunosuppressive therapy in organ transplant recipients for instance is held responsible for the occurrence of non Hodgkin s lymphomas and of epithelial tumours mostly originating from the cervix of the uterus and the skin (14) but also for the occurrence of acute leukemia (11). The incidence of malignant diseases in this population is 100 times higher than would normally be expected (8) which suggests a relationship between immunosuppressive drugs and second malignancy.

Proposed mechanisms behind the induction of malignancy are chromosome breakage (somatic mutation) activation of a latent oncogenic agent (virus) inability of identifying and destroying mutant cells and marrow aplasia (15).

In recent years the immunosuppressive drug azathioprine has been increasingly used in the treatment of non malignant conditions like systemic lupus erythematosus (SLE) rheumatoid arthritis dermatomyositis immune thrombocytopenia hemolytic anemia Crohn s disease and others. This therapy entails several serious hazards like thrombocytopenia leukopenia susceptibility to opportunistic infections and possibly induction of malignancy.

Evidently the benefits of such a treatment should be firmly established before a patient is exposed to its risks. We report a patient who died from subacute myelomonocytic leukemia (SMML) after five years treatment with azathioprine for SLE.

## CASE REPORT

In 1970 the patient was a 30-year-old housewife who had suffered for several years from periodic fever and arthralgias. In 1971 she developed a nephrotic syndrome with a proteinuria of 8 g/24 h. A renal biopsy showed membranous glomerulonephritis with proliferation and positive immunofluorescence with a granular pattern. A test for antinuclear factors and the LE cell test were positive. SLE was diagnosed on these findings.

Treatment was instituted with prednisone and azathioprine orally. The patient s condition improved fever arthralgias and proteinuria disappeared and the renal function remained normal (Fig 1). In 1973 azathioprine was withdrawn for 2 months because of neutropenia. A bone marrow preparation at that time showed no abnormalities except for iron deficiency. Hb concentration became normal after iron substitution but a slight thrombocytopenia persisted. Symptoms of SLE did not recur during this period. Azathioprine was reinstituted. A severe azathioprine attributed pancytopenia developed

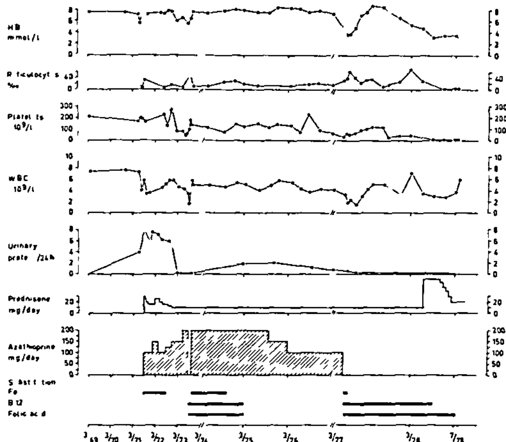



*Fig 1* Changes in Hb reticulocytes platelets WBC and urinary protein during treatment with azathioprine prednisone Fe $B_{12}$ and folic acid

1977 During this period the peripheral blood contained a few blast like cells Increased and atypical erythropoiesis and megakaryopoiesis and about 25% ringed sideroblasts were observed in the marrow Azathioprine administration was discontinued The anemia and leukopenia disappeared but again a slight thrombocytopenia persisted

In 1978 the pancytopenia recurred and the patient was admitted to hospital in April of that year Signs and symptoms that could be attributed to SLE were not present Except for pallor and multiple bruises physical examination was unremarkable Hepatosplenomegaly and lymphadenopathy were absent Hb was 3 7 mmol/l with a MCV of 120 fl The reticulocyte count was 67‰ the platelet count 7 5×$10^9$/l the WBC 4 3×$10^9$/l A smear of the peripheral blood showed an abnormal red blood cell picture with anisocytosis poikilocytosis and several erythroblasts About 30% abnormal monocytes and sporadic blast cells were found (Fig 2) The LAP index was 100 The ANA was positive the LE cell test negative and antibodies to DNA were not detected A bone marrow aspiration yielded cellular material (Fig 2) The granulopoiesis showed a shift to the left with an increase of blast cells (about 10%) Abnormal monocytoid cells were numerous The erythropoiesis was abnormal with megaloblastoid features multinuclearity and shift to the left Megakaryocytes were increased and highly abnormal showing dwarf forms hypo- and mononuclearity and shift to the left Erythrophagocytosis could be observed in several (large) blast cells (megakaryoblasts?) The number of mitoses was increased as was the number of macrophages Several abnormal sideroblasts and a small number of ringed sideroblasts were observed in the iron stain Peroxidase and α naphthyl butyrate esterase reactions were positive in some of the blast cells but negative in the majority Chromosomal abnormalities in cultured bone marrow cells were not observed The needle biopsy (Yamshidi) of the bone marrow showed a myeloproliferative process compatible with myelomonocytic leukemia. The serum lysozyme concentration was increased (34 μg/ml normal below 11) A diagnosis of SMML was made

The patient was treated symptomatically with 60 mg prednisone daily red cell and platelet transfusions She died from massive bleeding 2 months after diagnosis aged 38 At post mortem examination large quantities of blood were found in the peritoneal and retroperitoneal spaces and in the pleural and pericardial spaces Microscopical examination confirmed the diagnosis of SMML

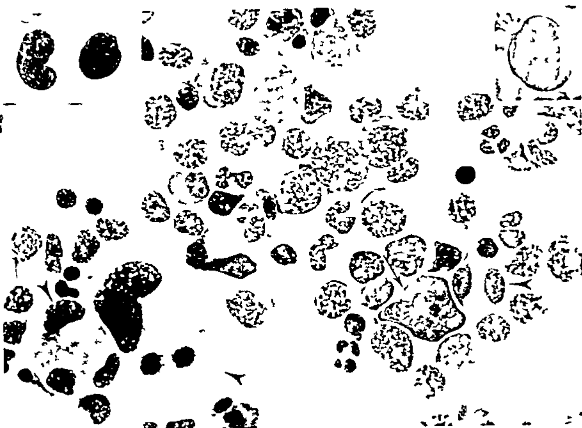


*Fig 2* L ght microscopy of the bone marrow (1977) showing abnormal megakaryocytes (*arrow heads*) a multinuclear erythroblast (*arrow*) and myelomonocytic prol feration *Inset* blast cell and abnormal monocytes of the peripheral blood

## DISCUSSION

This patient was treated for SLE and a nephrotic syndrome with prednisone and azathioprine She received azathioprine in a total dose of 273 g Seven years after in tiation and one year after discontinuation of this therapy SMML was diagnosed from which she died two months later The factors confirming the diagnosis of SMML were peripheral pancytopenia monocytosis abnormalities of three cell l nes (granulo- erythro- and megakaryopoiesis) and excess of blast cells in the cellular bone marrow cytochemical reactions and elevated serum concentration of lysozyme (19 ?5 26) We believe that this is the first case of SMML reported to occur after treatment with azathioprine

The occurrence of the leukemia in this patient might have been an event without any causal relationship with her underlying disease or the therapy given for it In fact an extensive study of SLE patients did not reveal a higher incidence of leukemia or one of the lymphoreticular malignancies in these patients than in the general population (25) However several arguments can be found supporting a causal relationship between the azathioprine therapy and the occurrence of leukemia Firstly the incidence of all malignant diseases in immunosuppressed patients appears to be 100-fold higher than expected (11) Secondly we found 16 other patients (1 2 3 4 6 9 11 16 17 18 ?0 21 22) with non lymphocytic leukemia after azathioprine treatment for non malignant disorders (Table I) Th rdly myelomonocytic leukemia is primarily a disease of patients over 50 years of age (23) while 4 out of 5 patients who contracted this type of leukemia after treatment with azathioprine (Table I) were aged 16 25 ?8 38 years Definite proof of a causal relationship is lacking especially in patients treated with other cytotoxic agents but the evidence is suggestive

Table I *Seventeen patients with non lymphocytic leukemia after azathioprine treatment*

SLE=systemic lupus erythematosus RA=rheumatoid arthritis SLN=subacute liver cell necrosis RT=renal transplant CGN=chronic glomerulonephritis NS=nephrotic syndrome AMML=acute myelomonocytic leukemia AML=acute myelogenous leukemia AMOL=acute monoblastic leukemia CML=chronic myelocytic leukemia SMML=subacute myelomonocytic leukemia P=corticoids Act D=actinomycin D C=cyclophosphamide G=gold Pe=penicillamine MTX=methotrexate Mit=mitoxine MP=mercaptopurine TBI=total body irradiation

| Age at onset of leukemia (y) | Sex | Diagnosis | Duration of azathioprine treatment (mo) | Total dose (g) | Months between start of azathioprine and diagnosis of leukemia | Other treatment | Type of leukemia | Reference no |
|---|---|---|---|---|---|---|---|---|
| 16 | ♀ | SLE | 66 | ? | 66 | – | AMML | 17 |
| 25 | ♂ | RA/SLE | 10 | 52 | 10 | Pe | AMML | 2 |
| 38 | ♀ | SLE | 65 | 273 | 77 | P | SMML | Present paper |
| 73 | ♀ | RA | 40 | 95 | 40 | P | AML | 9 |
| 57 | ♀ | RA | 30 | 109 | 37 | P C G | AML | 18 |
| 68 | ♂ | RA | 65 | 255 | 67 | P | AML | 18 |
| 67 | ♂ | RA | 24 | 77 | 24 | P C MTX Mit | AML | 6 |
| 23 | ♂ | SLN | 36 | 156 | 38 | P | AML | 21 |
| 51 | ♂ | RT | 145 | 400 | 150 | MP TBI 360 rad | AMOL | 11 |
| 22 | ♂ | RT | 26 | 103 | 26 | P | CML | 1 |
| 32 | ♂ | RT | 46 | 113 | 46 | P + Act D | AML | 21 |
| 47 | ♂ | RT | 5½ | 19 2 | 21 | – | AML | 22 |
| 37 | ♂ | RT | 56 | ? | 57 | – | AML | 20 |
| 38 | ♀ | RT | 57 | ? | 57 | – | AML | 20 |
| 53 | ♂ | RT | 37 | 60 | 36 | MP | AMML | 4 |
| 15 | ♂ | CGN | 29 | 4–5 mg/kg/d | 49 | – | CML | 3 |
| 28 | ♂ | NS | 84 | ? | 60 | C | AMML | 16 |

Azathioprine is an effective immunosuppressive agent Its administration to patients with organ transplants appears to be fully justified and its potential to induce malignant changes may be acceptable as a calculated risk However the beneficial effects of this drug in the treatment of SLE is questionable (7 10) There is no convincing evidence that the dose of corticosteroids necessary to control this disorder is lower when azathioprine is added to the therapeutic regimen (24) Our patient received a daily dose of 100–200 mg azathioprine continuously for six years with an interruption of two months in combination with 10 mg of prednisone

We consider that the risks of azathioprine in the treatment of SLE are acceptable only if repeated trials in an individual patient prove that there is a substantial benefit from the drug for that particular patient

## REFERENCES


1 Adler R K Lempert N & Schartman W B Chronic granulocytic leukemia following successful renal transplantation Cancer 41 2206 1978
2 Alexson E & Brandt K D Acute leukemia after azathioprine treatment of connective tissue disease Am J Med Sci 273 335 1977
3 Battin J Henunstre J P Bui N B et al Leucemie myeloide chronique (L M C) apres traitement immunodepresseur pour néphropathie chronique Nouv Presse Med 5 2632 1976
4 Blanc A P Gastaut J A Dalivoust P et al Hemopathies malignes survenant au cours d un traitement immunosuppresseur Nouv Presse Med 6 2503 1977
5 Brody R S Schottenfeld D & Reid A Multiple primary cancer risk after therapy for Hodgkin s disease Cancer 40 1917 1977
6 Cobau C D Sheon R D & Kirsner A B Immunosuppressive drugs and acute leukemia Ann Intern Med 79 131 1973
7 Decker J L Klippel J H Plotz P H et al

Cyclophosphamide or azathioprine in lupus glomerulonephritis Ann Intern Med 83 606 1975
8 Editorial Cancer in the immunosuppressed patient Ann Intern Med 75 310 1971
9 Gilmore I T & Holden G Acute leukaemia during azathioprine therapy Postgrad Med J 53 173 1977
10 Ginzler D Sharon E Diamond H et al Long term maintenance therapy with azathioprine in systemic lupus erythematosus Arthritis Rheum 18 27 1975
11 Grunwald H W & Rosner F Acute leukemia and immunosuppressive drug use a review of patients undergoing immunosuppressive therapy for non neoplastic diseases Arch Intern Med 139 461 1979
12 Kyle R A Pierre R V & Bayrd E D Multiple myeloma and acute leukemia associated with alkylating agents Arch Intern Med 135 185 1975
13 Oleinick A Leukemia or lymphoma occurring subsequent to an autoimmune disease Blood 29 144 1967
14 Penn I Halgrimson C G & Starzl T E De novo malignant tumors in organ transplant recipients Transplant Proc III 773 1971
15 Potolsky A & Cregers W D Radiation and drug therapies and leukemia Annu Rev Med 24 75 1973
16 Roberts M M & Bell R Acute leukemia after immunosuppressive therapy Lancet 2 768 1976
17 Rosner F & Grunwald H W Multiple myeloma terminating in acute leukemia Am J Med 57 927 1974
18 Seidenfeld A M Smythe H A Ogryzlo M A et al Acute leukemia in rheumatoid arthritis treated with cytotoxic agents J Rheumatol 3 295 1976
19 Sexauer J Kass L & Schnitzer B Subacute myelomonocytic leukemia. Am J Med 57 853 1974
20 Sheil A G R Cancer in renal allograft recipients in Australia and New Zealand Transplant Proc 9 1133 1977
21 Silvergleid A J & Schrier S L Acute myelogenous leukemia in two patients treated with azathioprine for nonmalignant diseases Am J Med 57 885 1974
22 Sloan G M Cole P & Wilson R E Risk indicators of denovo malignancy in renal transplant recipients Transplant Proc 9 1129 1977
23 Sultan D Dysmyelopoietic syndromes In Classification of acute leukemia (moderator H R Gralnick) pp 749–752 Ann Intern Med 87 740 1977
24 Swaak A J G Status van Eps L W & Feltkamp T E W Clinical management of SLE patients Neth J Med 21 44 1978
25 Zittoun R Subacute and chronic myelomonocytic leukaemia a distinct haematological entity Br J Haematol 32 1 1976
26 Zittoun R Certin M Audebert A et al Evolution des anemies refractaires Rapports avec les leucemies myelomonocytaires Ann Med Interne 125 593 1974
27 Zwaan F E den Ottolander G J Brederoo P et al The morphology of dyserythropoiesis in a patient with acute erythroleukaemia associated with multiple myeloma Scand J Haematol 17 353 1976

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# Predicting Response to Combination Chemotherapy in Acute Myeloblastic Leukemia—a Way to Individualize Treatment

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ABSTRACT Cytostatics are toxic, and individual variations in drug metabolism large Therefore the possibility to predict which cytostatic combination should be abandoned because it will not lead to a remission was studied in 58 leukemia patients A simple following of changes in white blood cell, platelet and blast cell numbers in peripheral blood (combined into so-called chemotherapy points) makes it possible to predict which patients in the presenting phase of AML will achieve remission, but this was possible neither in the relapse phase of AML nor in promyelocytic or monocytic leukemias All 27 patients in the initial phase of AML, who later were to achieve remission, had less than 12 chemotherapy points, and 9 of 11 patients who did not achieve remission had over 13 points Therefore the cytostatic combination should be changed in patients who, after two courses of treatment have over 13 points

*Key words* acute myeloblastic leukemia chemotherapy individualized treatment

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There are few drugs which are more toxic than cytostatics and there are no observations indicating that individual variations in drug metabolism and drug tolerance are smaller for cytostatics than for other drugs (1 2 3 4 7 8) For this reason it becomes important to individualize treatment with cytostatic drugs (6) However the present trend is rather than to individualize treatment to randomize patients to receive one of several standardized dose chemotherapy combinations for a standardized period (5)

In the Leukemia Group of Central Sweden a minimum of 4 and a maximum of 6 courses of a combination of rubidomycin and cytosine arabinoside are given to every patient prior to change of treatment (5) Marrow aplasia can postpone a course of treatment but not change the cytostatic combination or the dose It must be assumed that such standardization of treatment will result in some patients in too short or too weak treatment in others in too long or too strong A number of alternative drug combinations exist leading to about 60% complete remissions and patients may well be refractory to one and respond to another drug combination

The purpose of this study was to find out if by carefully following peripheral blood values it would become possible to predict which patients are going to respond and which are refractory to a particular form of treatment so that the chemotherapy combination or the dose can be changed earlier than after 4–6 courses of treatment This number of courses usually requires 1–3 months of treatment and during this time some patients may actually die of granulocytopenia and septicemia, or of bleeding

## PATIENTS

Of all acute leukemias admitted to the Division of Hematology from 1972 to May 1978 58 patients were studied in three groups Group 1 comprised 38 patients

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*Abbreviations* AML = acute myeloblastic leukemia WBC = white blood cells C

Table I *Cytostatic combinations in the initial phase and in relapse of AML*

Since all patients received cytosine arabinoside and thioguanine as maintenance in remission only 2 were given completely new cytostatics in relapse

| | No of pats | |
|---|---|---|
| | Initial phase | Relapse |
| Cytosine arabinoside and rubidomycin | 24 | 2 |
| Cytosine arabinoside and thioguanine | | 6 |
| Vincristine methotrexate 6-mercaptopurine and prednisolone | | 2 |

Table II *Leukemic blast cell reappearance in peripheral blood after chemotherapy*

| | No of pats | | |
|---|---|---|---|
| | A pattern (blast decrease or increase <50%) | B pattern (blast increase >50%) | % of pats with B pattern |
| Initial phase | | | |
| Remission | 22 | 5 | 18 5 |
| No remission | 7 | 4 | 36 3 |
| Relapse | | | |
| 2nd remission | 1 | 2 | 66 6 |
| No 2nd remission | 6 | 1 | 14 2 |

with acute myeloblastic leukemia (AML) in the initial phase Group 2 comprised 10 patients with AML in relapse and group 3 6 with promyelocytic and 4 with monocytic leukemia Leukemias preceded by a preleukemic state were excluded but no other patients

## METHODS

Patients were followed with blood counts performed before each course of chemotherapy and 4–6 days after the final day of each course of chemotherapy Only total white blood cell (WBC) counts platelet counts and differential counts were used all performed by routine hematological methods We attempted to combine postchemotherapy changes (for details see below) in both total WBC platelets and leukemic blast cells into one figure called chemotherapy points (CTP) which could then be compared to the therapeutic results A favourable response to treatment was given 1 CTP unfavourable 3 CTP Several different transformations of the blood counts were tested The one found best correlated to the prognosis the one which can best predict if the patient is going to respond or not is described below

An increase in WBC after chemotherapy or a total number over 400/mm³ was awarded 1 CTP no increase in WBC or a total number of 150–400/mm³ 2 CTP and a total number under 150/mm³ 3 CTP The values after the first course of treatment were compared to those before treatment those after the second course to those immediately before the second course An increase in platelets or a total number over 75 000/mm³ was given 1 CTP a total number of 10 000–75 000 2 CTP and less than 10 000/mm³ 3 CTP

A decrease in the number of leukemic blasts exceeding 60% of the initial blast number or fewer blasts than 8% of the total WBC were awarded 1 CTP a blast decrease of 50–60% 2 CTP and a blast decrease of less than 50% or a blast increase 3 CTP All these points were calculated after the first and second course of treatment The CTP for side-effects i e leukopenia and thrombocytopenia after the first two courses of chemotherapy were added and the sum was multiplied by the CTP for antineoplastic effect i e the effect on the blast cells The reappearance of leukemic blasts in the peripheral blood after chemotherapy was named blast plattern *A* if the percentage of blasts increased by less than 50% of the value after the first 2 courses of treatment within 8–12 days and blast pattern *B* if the blast reappearance was more rapid

Complete remissions were defined as a complete normalization of the peripheral blood picture Qualitative studies of the bone marrow smears and sections in complete remission revealed no myeloblast increase suggesting that as a rule the myeloblast count was lower than 5% Partial remissions were defined as a complete normalization of the peripheral blood picture with remaining apparent increases in the bone marrow cellularity

All blood counts and differential counts were performed by one or two technicians who work almost exclusively with pathological differential counts Doubtful cells were studied by one of us (P R) We believe that the reporducibility of such a system is necessary for findings of the present nature which are probably more difficult to detect if numerous technicians in a large central labora ory divide the work between them

## RESULTS

### *Group 1 Chemotherapy points and chemotherapy response in the initial phase of AML*

This group includes only patients in the presenting phase of AML Patients in relapse and patients with promyelocytic monocytic and myelomonocytic leukemia were excluded Under these conditions the CTPs found at the end of the second course of treatment seemed to predict remission with a certain accuracy All 27 patients who later were to achieve a complete remission had less than 12 CTP whereas 9 out of 11 patients who were not to

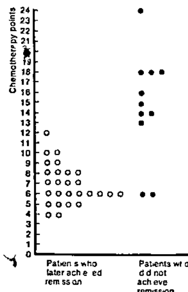



*Fig 1* Group 1 initial phase of AML CTPs after the second chemotherapy course in patients later achieving complete remission (O) and in those who died without remission (●)

achieve complete remission had more than 13 CTP (Fig 1)

### *Group 2 CTP and response to chemotherapy in AML in relapse*

In contrast no agreement could be found in relapse between the CTP and the end of the second chemoterahpy course and the response to treatment All 10 patients in relapse had 13 CTP or less

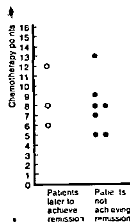



*Fig 2* Group 2 AML in relapse CTPs after the second chemotherapy course during relapse in patients who achieved a second remission (O) and in those who did not (●)

Table III *Chemotherapy points after chemotherapy in patients with a slow A pattern and a rapid B pattern reappearance of leukemic blast cells in the peripheral blood*

| | *A* pattern | | | *B* pattern | | |
|---|---|---|---|---|---|---|
| | No of pats | CTP Mean | CTP Range | No of pats | CTP Mean | CTP Range |
| Initial phase | 22 | 7 18 | 4–12 | 5 | 6 2 | 5–7 |
| Relapse | 7 | 6 85 | 5–9 | 3 | 11 0 | 8–13 |

although only 3 of them achieved a complete second remission (Fig 2) A cytostatic combination different from that used during the initial phase of the disease was used in relapse in 8 of the 10 relapsed patients However only 2 of these 8 patients received cytostatics which had not already been used for remission maintenance (Table I)

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### *Group 3 Patients with promyelocytic and monocytic leukemia*

The 10 patients included in this group are described in Fig 3 In these patients the CTP permitted no conclusion regarding complete remission

## DISCUSSION

It would perhaps have seemed more scientific or at least more erudite to use marrow differential counts before and after each course of treatment rather than the simple blood counts used here The latter were chosen for two reasons First repeated marrow punctures are more laborious and painful than blood counts Second there was so little overlapping between the two groups (Fig 1) identified with the present simple methods that little could be gained by more refined methods Nevertheless the latter should be tried also

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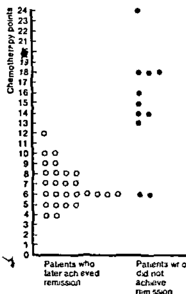



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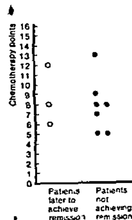



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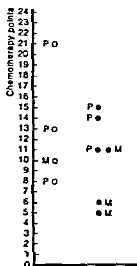



Fig. 3 Group 3 CTPs in patients with acute promyelocytic (*P*) and monocytic leukemia (*M*) who did (O) and who did not achieve remission (●)

It is not surprising that many of the patients whose blasts disappear early during treatment but whose leukocytes and platelets do not disappear altogether achieve a remission Nor is it surprising that relatively few do of those who show disappearing normal cells and remaining blasts However it is surprising that this relationship could be found only for the acute myeloblastic leukemias and not for the promyelocytic and monocytic leukemias All morphological diagnoses were made prior to and independently of the present analysis which was always made in retrospect This unexpected finding requires further study perhaps by marrow differential counts rather than the present simple blood counts

The predictive value of following the peripheral blood values of the chemotherapy seems to be appreciably smaller in patients with acute leukemia in relapse than in those in the initial phase of the disease All of the 10 patients studied in relapse had peripheral blood value reactions which in the initial phase of the disease would have indicated a remission However only 3 out of the 10 patients did achieve a second remission The present results are insufficient to indicate whether this finding is due to drug resistance in relapse or to a change in the disease process itself for instance tumor progression It seems most desirable to perform studies able to distinguish between these two alternatives if more frequent second remissions in acute leukemia are to be achieved

If the present results are confirmed it seems to become possible to discontinue chemotherapy combinations producing unfavourable peripheral blood reactions in an individual patient at an early stage and to replace them by hopefully more efficient combinations It should also eventually become possible to obtain guidelines regarding the dosage of cytostatics The possibility should be studied whether very low CTP for granulocytes and platelets would permit an increase in dose and whether very high CTP for granulocytes and platelets would if the CTP for leukemic blast cells is low suggest a decrease in dose Obviously if the CTP is very high for all three the cytostatic combination should be changed It does seem possible to use potentially very toxic cytostatic combinations in the future rather than a standard dose in all patients with acute leukemia to give every patient a relatively moderate test dose of a cytostatic combination and to adjust the future treatment according to the changes in the peripheral blood values Such a procedure might become valuable even when long term cytostatic combinations in high doses are used initially which result in a high incidence of complete remission even after the first course of treatment Even after the first course of combination chemotherapy valuable indications may sometimes be found by studying the peripheral blood values which indicate the future course of the disease with this particular combination

## ACKNOWLEDGEMENT

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## REFERENCES

1 Andersson B Beran M Eberhardsson B Eksborg S & Slanina P Uptake and distribution of daunorubicin and daunorubicin DNA complex in mice as studied by whole body autoradiography and liquid chromatography Cancer Chemother Pharmacol 2 159 1979
2 Asbell M A Schwartzbach E Wodinsky I & Yesair D W Metabolism of daunomycin (NSC 82151) in vitro and the chemotherapeutic activity of isolated metabolites in vivo Cancer Chemother Rep Part 1 56 315 1972
3 Beran M Andersson B Eksborg S & Ehrsson H Comparative studies on the in vitro killing of human normal and leukemic clonogenic cells (CFUc)

by daunorubicin daunorubicinol and daunorubicin DNA complex Cancer Chemother Pharmacol 2 19 1979
4 Eksborg S Ehrsson H Andersson B & Beran M Liquid chromatographic determination of daunorubicin and daunorubicinol in plasma from leukemic patients J Chromatogr 153 211 1978
5 Gahrton G Engstedt L Franzén S et al Induction of remission with L asparaginase cyclophosphamide cytosine arabinoside and prednisolone in adult patients with acute leukemia Cancer 34 472 1974
6 Johansson N O & Reizenstein P Predicting efficacy of chemotherapeutic combinations in acute leukemia—a way to individualize treatment Trans Int Soc Haematol Eur Afr Div Abstr p 251 1977
7 Niho Y Till J E & McCulloch E A Effect of arabinocyl cytosine on granulopoietic colony formation by marrow cells from leukemic and non leukemic patients Exp Hematol 4 63 1976
8 Salmon S Hamburger A W Soehnlen B Durie B G M Alberts D S & Moon T E Quantitation of differential sensitivity of human tumor stem cells to anticancer drugs N Engl J Med 298 1321 1978

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SHORT COMMUNICATION

# Comments on the Nomenclature when Describing May–Grunwald–Giemsa-Stained Bone Marrow Smears

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In a recent issue of Clinics in Haematology Professor Lajtha (3) recalled the long gone days when haematologists argued the identity of relatively featureless cells and how things have changed since then with a series of new methods for quantitative analyses of operationally defined subpopulations of the haematopoietic cells What Lajtha says about all the new methods is undoubtedly correct but the need to argue the identity of immature cells in May–Grunwald–Giemsa (MGG)-stained bone marrow films unfortunately still remains Clinicians who have to decide whether a patient has leukaemia or not and which type of leukaemia are daily forced to describe the immature cells in a MGG stained bone marrow smear Evaluation of a properly prepared MGG stained bone marrow is one of the first and still probably the most important step in establishing the diagnosis and type of leukaemia

The cellularity and the percentage of nucleated cells belonging to the respective cell lines and stages of development are estimated and compared to the percentage found in normals It is therefore important that we know the normal values and use the same criteria to define the different stages of the different cell lines

The most immature looking cells in the bone marrow have scanty pale blue cytoplasm without granules finely dispersed nuclear chromatin and nucleoli A normal bone marrow contains only a few per cent of such cells and it is very difficult if not impossible to know whether a given cell is a pluripotent haemocytoblast or is on its way to becoming a granulocyte or a lymphocyte In acute leukaemia on the other hand a large number of such immature cells is usually found in the bone marrow If more than about 3% of the immature cells show azurophilic granules (which also are Sudan black B positive) or if Auer rods are found it may safely be assumed that the patient has acute myeloid leukaemia

In acute myeloid leukaemia most of the immature cells without granules or Auer rods will be on their way to becoming granulated (although a large number will never arrive!) In American and North West European tradition these non granulated immature cells in acute myeloid leukaemia are called myeloblasts In French and Italian tradition however the word myeloblast is not used of cells which do not have granules or Auer rods in MGG stained smears

All immature cells with nucleoli basophilic cytoplasm and azurophilic granulation are called promyelocytes in American and North West European tradition In French and Italian tradition however the most primitive of these granulated immature cells are called myeloblasts and only the less primitive ones are called promyelocytes

When French and Italian authors publish papers or books in English but keep their traditional nomenclature one may easily be confused To hope for an international consensus unifying the nomenclature would probably be too optimistic knowing human nature Switching from one set of well established criteria to the other might be as painful as switching between driving on the right or left side of the road One less difficult way of solving some problems however would be if those using the American and North West European criteria would omit the word myeloblasts for non granulated immature cells and instead use the non-committal word blasts a practice which some workers have in fact adopted The users of the French and Italian criteria would on their side reduce the differences if they always would list myeloblasts and promyelocytes together and preferably add the word granulated in parentheses after

myeloblasts I would think that the distinction between myeloblasts (granulated) and promyelocytes is rather diffuse anyway

The FAB classification (1) defines one type of acute myeloid leukaemia as hypergranular promyelocytic (M3) and the characteristic features are numerous rather coarse granulae and usually many Auer rods in a high percentage of the immature cells A striking feature in these patients is uncompensated DIC resulting in severe bleeding tendency Most French American and British workers who use the diagnosis acute promyelocytic leukaemia probably are referring to patients with this hypergranular form Many German workers on the other hand use the Loeffler classification in which acute promyelocytic leukaemia also includes patients whose promyelocytes have fine and sparse granulae and few if any Auer rods (2) These patients do not have the characteristic DIC and the bleeding tendency of the hypergranulated ones

Comparing acute promyelocytic leukaemia according to Loeffler's criteria with acute hypergranular promyelocytic leukaemia according to the FAB classification will therefore be confusing Such a confusion can easily be avoided if those who are referring to acute hypergranular promyelocytic leukaemia do not omit the important word hypergranular (1)

## REFERENCES


1 Bennet J M Catovsky D Daniel M T Flandrin G Galton D A G Gralnick H R & Sultan C Proposals for the classification of the acute leukaemias by French American British (FAB) Co-operative Group Br J Haematol 33 451 1976

2 Fulle H H Gruneisen A Karow J Koeppen K M Oertel J Ruhl H & Schwerdtfeger R Prognostic relevance of cytochemical classification of adult acute leukemias Abstracts V International Soc of Haematol Europ and African Div 5th meeting Hamburg 1979

3 Lajtha L G Cellular dynamics of haematopoiesis foreword Clin Haematol 8 219 1979

# Spontaneous Pneumothorax as First Symptom in Bronchial Carcinoma

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ABSTRACT Pneumothorax is a rare manifestation of lung cancer It has been reported to date in a total of about 25 patients We describe two patients with spontaneous pneumothorax as the first sign of a bronchial carcinoma

*Key words* bronchial carcinoma spontaneous pneumothorax

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Pneumothorax as the presenting symptom in bronchial carcinoma has seldom been described in the literature Mahajan et al (4) found only 20 cases in the English literature and also described one case of their own Going through the records of 1 143 patients with spontaneous pneumothorax treated at the Mayo Clinic during a 20-year period Dines et al (1) found only four cases of bronchial carcinoma According to Hyde (2) about 25 patients with pneumothorax and lung cancer have been reported up to date We will describe two patients with primary bronchial carcinoma whose first symptoms were those of spontaneous pneumothorax

## CASE REPORTS

### *Case I*

A 72 year-old man office employee He had been a moderate smoker for many years but had stopped smoking 20 years ago For many years he had had symptoms of chronic bronchitis with cough and sputum

The patient was admitted to the hospital in Jan 1974 with a history of about two months of increasing dyspnea but no chest pain On physical examination he was quite unaffected and without dyspnea at rest There was no clubbing of the fingers The right hemithorax revealed poor breath sounds and a diminished percussion note in the basal parts Examination of the other organs revealed no abnormality Routine blood and urine laboratory tests were normal

Chest X ray (Fig 1) showed a moderate pneumothorax and a small amount of pleural fluid on the right side In the right lower lobe close to the fissure between lower and upper lobe a rounded tumour with a diameter of 1 cm was seen Thoracoscopy revealed small yellowish areas in the parietal pleura while the visceral pleura seemed macroscopically normal Biopsy from the pathologically changed parietal pleura showed atypical cells suspected of malignancy Cytological examination of the pleural fluid also revealed atypical malignant cells Bronchoscopy was normal and so was cytological examination of sputum and bronchial secretions

Even with pleural drainage it was not possible to eliminate the patient s pneumothorax He was therefore admitted to the surgical ward and operated upon Thoracotomy revealed a tumour in the fissure between the lower and upper lobes on the right side and tumour growth in both visceral and parietal pleura Histopathological examination showed a low differentiated squamous cell carcinoma

Treatment with chemotherapy was instituted but no improvement was seen The patient died eight months later

### *Case II*

A 71 year-old man a retired butcher who stopped smoking four years ago During the last 30 years he had had symptoms of chronic bronchitis and emphysema with cough sputum and dyspnea In 1974 he received respirator treatment because of bronchopneumonia with acute respiratory failure In Feb 1978 he became acutely ill with intense chest pain and breathlessness

On physical examination he was dyspnoic and cyanotic Chest X ray showed a large pneumothorax with total collapse of the right lung The pneumothorax was treated with a pleural drainage Chest X ray revealed a remaining atelectasis of the middle lobe after the pneumothorax had been removed

Even with pleural drainage it was not possible to eliminate the pneumothorax and thoracotomy was performed showing a large emphysematous bulla with a small punched hole The bulla was resected Sputum cytology showed suspected malignant cells and later fiberoptic bronchoscopy verified a squamous cell carcinoma occluding the middle lobe bronchus

Radiotherapy was not instituted because of poor lung function Treatment with chemotherapy gave no improvement and the patient died nine months later



*Fig 1* Roentgenogram (case I) showing a pneumothorax and a small amount of pleural fluid on the right side



Between the upper and lower lobes of the right lung there is a small tumour (arrow)

## DISCUSSION

In the age groups where bronchial carcinoma is most frequent spontaneous pneumothorax is rather unusual The combination of bronchial carcinoma and spontaneous pneumothorax is rare (1 2 4) but when found it is ipsilateral This suggests that the two diagnoses are connected when they occur simultaneously Another indication of such a cause–effect relationship is the occurrence of spontaneous pneumothorax in children with pulmonary metastases from bone tumours (5) Spontaneous pneumothorax in children is otherwise very uncommon

Various mechanisms have been suggested for the occurrence of pneumothorax in bronchial carcinoma According to Killen and Gobbel (3) one mechanism could be bronchial obstruction by a centrally placed tumour with local emphysema in the periphery In these cases rupture of the emphysematous cysts to the pleura could lead to spontaneous pneumothorax Our case II illustrates this mechanism well If bronchial obstruction does occur there could also be an infection with abscess formation distal to the tumour with perforation to the pleural cavity Another possibility could be that the tumour itself grows into the visceral pleura Eventually necrosis develops in the tumour and leads to the formation of a bronchopleural fistula with spontaneous pneumothorax This is illustrated by our case I who had a rather small and peripherally located tumour The tumour had grown through the visceral pleura and thus given rise to spontaneous pneumothorax as well as widespread metastases in the pleural cavity

We believe that bronchial carcinoma should always be considered as a possible cause of spontaneous pneumothorax in patients especially in smokers in the older age groups

## REFERENCES

1 Dines D E Cortese D A Brenner M D Hahn R G & Payne W Malignant pulmonary neoplasms predisposing to spontaneous pneumothorax Mayo Clin Proc 48 541 1973

2 Hyde L Pneumothorax A rare manifestation of lung cancer Chest 72 557 1977

3 Killen D A & Gobbel W G Spontaneous pneumothorax p 108 Little Brown & Co Boston 1968

4 Mahajan V Kupferer C F & Van Ordstrand H S Pneumothorax A rare manifestation of primary lung cancer Chest 68 730 1975

5 Spittle M F Heal J Harmer C & White W F The association of spontaneous pneumothorax with pulmonary metastases in bone tumours in children Clin Radiol 19 400 1968

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# Two Patients with Recurrent Pneumonias in One Lung

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ABSTRACT Case histories are described of two young patients, both known to have recurrent infections of one pulmonary lobe Further investigations (especially aortography) disclosed the cause in both to be an intrapulmonary sequestration Surgical intervention made both patients symptom free The aetiology, clinical and investigational findings as well as therapy are discussed The need for further investigations in patients with recurring infections of one pulmonary lobe is stressed

*Key words* intrapulmonary sequestration recurrent pulmonary infections

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Two patients are reported who for some years were known to have recurrent infections of one pulmonary lobe Both have frequently been treated with antibiotics without further aetiological investigations A rather infrequent congenital abnormality was found to be the underlying cause Surgical intervention was performed and since then both patients have been asymptomatic

## CASE REPORTS

*Case I* (Fig 1)

A 14-year-old boy was seen in Feb 1978 He was known to have recurrent infections of the lower lobe of the left lung since shortly after birth Treatment with antibiotics has always been successful but since Nov 1977 the episodes occurred more frequently

On physical examination a slender boy was seen with a rectal temperature of 38 4 C Chest examination disclosed a softened percussion and moist rales as well as an amphoric respiration at the dorsum of the left lung Further examination showed no abnormalities

Laboratory findings ESR 17 mm in the first hour leucocytes 12 6×10⁹/l with a normal differentiation Blood and sputum cultures were sterile A chest X ray showed a shadow in the left lower lobe also visible on X rays taken of the preceding years Planigraphy revealed a sharply defined homogeneous shadow behind the heart and extending from the dorsal to the thoracic wall until halfway the left lower lobe Bronchography disclosed a relatively small left lower lobe in which no bronchial branches could be visualized in the dorso-basal segment Bronchoscopy did not show any abnormality

As a pulmonary sequestration was suspected a percutaneous aortography was performed at the level of the 10th thoracic vertebra an anomalous artery originating from the dorsal aorta was found which supplied the involved area Venous drainage was via the vena pulmonalis to the left atrium

An intrapulmonary sequestration was diagnosed and surgical resection was performed At operation the sequester and the basal segments of the left lower lobe were removed as the latter were infectiously involved A large artery originating from the aorta and lying within the pulmonary ligament supplied the area There were several large veins which debouched into the vena pulmonalis inferior There were many pleural adhesions The postoperative course was uneventful and the boy has been free from symptoms

Histological examination showed a sequester of 9×8×7 cm The hilus contained some elastic arterial branches The parenchyma disclosed broad fibrous septa peripherally widened bronchi filled with erythrocytes and mucus The alveoli were mostly filled with macrophages The pleura showed oedema of the connective tissue with swollen mesothelium

*Case II* (Fig 2)

A 7 year-old boy born in Turkey and living in the Netherlands for the last 4 years was admitted in Sept 1977 because of recurrent fever upper abdominal pain anorexia and nausea of 4 weeks duration He was reported to have had these episodes about 40 times since 5 months after birth

Physical examinations showed a well nourished boy with a rectal temperature of 37 8°C At the heart a second grade systolic murmur was heard (loudest at the second intercostal space at the right side) which was not propagated At percussion a softened sound at the right lower basal side was heard and at auscultation there were moist rales Further examination showed no abnormalities

Laboratory findings ESR 43 mm in the first hour leucocytes 8 1×10⁹/l with a normal differentiation Cultures of nose and throat swabs showed Staphylococci aurei and Diplococci pneumoniae respectively

A chest X ray showed an infiltration with cavities in the right lower lobe A right lower lobe pneumonia was diagnosed and therapy with ampicillin was initiated The patient soon became free from symptoms A control chest X ray disclosed resolution of the infiltrate but now differ

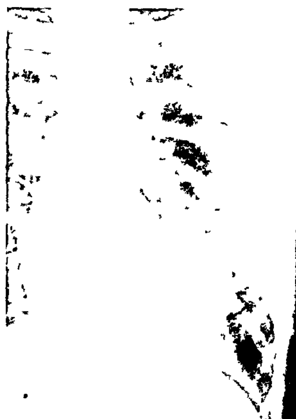






*Fig 1* Case I (*a*) Chest X ray note the shadow in the left lower lobe (arrow) (*b*) Bronchography showing no bronchial branches in the small left lower lobe (*c*) Aortography anomalous artery originating from the dorsal aorta supplying the left lower lobe

ent cavities were visible At bronchography no filling was found in the right lower lobe and the bronchial branch of the basal segments diverged around the affected are [illegible] percutaneous aortography a rather large artery was found which originated from the dorsal aorta at the level of the 10th thoracic vertebra and supplied the area in the right lower lobe Venous drainage was via the vena pulmonalis inferior to the left atrium

An intrapulmonary sequestration was diagnosed and thoracotomy was performed in Nov 1977 The sequester was removed There were extensive adhesions to the diaphragma and the pleura parietalis The sequester contained multiple cavities filled with mucus The supplying artery was embedded in the pulmonary ligament and had a diameter of 5 mm Several venous branches led to the vena pulmonalis inferior The postoperative course was uneventful and the boy has been free from symptoms

At histological examination the sequester was found to be 8×7×4 cm The bronchi were dilated and filled with mucus leucocytes and macrophages the alveoli were filled with foam cells The parenchyma had partly collapsed partly been replaced by fibrous tissue The interstitium showed a lympho-plasmocytic infiltration







*F g 2* Case II (*a*) Chest X ray note the cav t es wh ch were seen after resolut on of the nf ltrate (*b*) Bronchography no f ll ng of the r ght lower lobe and d vergence of the bronchial branches (*c*) Aortography anomalous ar tery orig nat ng from the dorsal aorta supply ng the right lower lobe

## DISCUSSION

A pulmonary sequestration is a congenital abnor mality in wh ch the involved area may rema n with in the lung (intrapulmonary sequestrat on) or be enclosed in its own pleural sac (extrapulmonary sequestration) (2 8) *Mostly there seems to be no* connect on with the bronchial tree The arterial blood supply orig nates from the aorta Although intermed ate forms do exist one m ght roughly state that 85% are intrapulmonary and 15% ex trapulmonary (4)

*I tr p l tary se j estrat i* (Table I) With n the pleural sac there is a non functioning part of the lung Blood supply is either from the dorsal aorta or one of its branches The anomalous artery is a per sisting branch of the splanchn c plexus wh ch most ly d sappears in the 8 mm embryonal stage A re v ew of the blood supply in 114 cases collected from

# ANNOUNCEMENTS

*XVII Congress of the European Dialysis and Transplant Association and IX Annual Conference of the European Dialysis and Transplant Nurses Association* will be held in Prague June 10–13 1980

*Information* Czechoslovak Medical Society, J E Purkyne Congress EDTA/Conference EDTNA 12026 Prague 2 Czechoslovakia.

*IX International Congress of the International Society of Psychoneuroendocrinology* will be held in Florence Italy June 16–20 1980

*Organizing Secretariat* Fondazione Giovanni Lorenzini Via Monte Napoleone 23 I 20121 Milan Italy

*Thirteenth Miles International Symposium* will take place at *Johns Hopkins Medical Institutions in Elkhart IN* USA June 18–20 1980 Topic of the symposium "Nutritional factors Modulating effects on metabolic processes"

*Further information* E G Bassett Ph D Symposium Coordinator Miles Laboratories Inc P O Box 40 Elkhart IN 46515 USA

*8th International Congress of Physical Medicine and Rehabilitation* will be held in Stockholm Sweden Au 25–29 1980 The congress will include seven main sessions ten symposia on current topics in the field of rehabilitation medicine free communications posters and films Official languages will be English French German and Spanish

*Further information* Congress Secretariat Physical Medicine c/o Stockholm Convention Bureau Jakobs Torg 3 S-111 52 Stockholm Sweden

*Fondazione Giovanni Lorenzini forthcoming scientific activities* International Symposium on Medical Statistics *Rome Italy Sept 22–24 1980 International Symposium* on New Trends in Antibiotics Research and Therapy Milan Italy Oct 29–31 1980

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REVIEW ARTICLE

# Sick Cell Receptors and Disease

During the last few years it has become clear that cell refractoriness against active substances hormones may simulate hypohormonal conditions A syndrome mimicking diabetes may be caused not only by insufficient secretion of insulin or by true circulating antibodies against this hormone but also thirdly by lack of active receptors on the surface of the target cells

The great American endocrinologist Fuller Albright coined the expression end organ refractoriness or the Seabright Bantam syndrome (1) Cocks of this race sometimes do not develop normal male plumage but look like hens in spite of the fact that they have male gonads The interesting thing is that they do not become male in appearance after injection with androgens Albright drew the conclusion that the testes produce normal sex hormones but that these steroids do not act on the end organs As a matter of fact not only the plumage but also the accessory sex organs do not develop normally

The concept of a refractory state in some cells was used by Albright to explain a rare but interesting human disease when a patient develops tetany in spite of normal parathyroid glands These patients are also refractory to the injection of parathormone This condition is usually congenital and may of course be caused by deficient formation of receptors for parathormone Other explanations are possible however See next paper

A group of investigators in DeLuca s laboratory has recently analyzed why patients with such pseudohypoparathyroidism are refractory to the hormone They seem to have proved conclusively that the defect lies in a deficient formation of 1 25 (OH) 2D the active metabolite of vitamin D When this substance was given serum calcium increased and serum PTH decreased It is thus probable that this is—at least in some patients—an enzymic defect like most inborn errors of metabolism and the [illegible] of end organ refractoriness sick receptors [illegible]as probably not correct in the first instance of a human disease where it was applied

A beautiful *true* example of this mechanism is the inherited disease testicular feminization (TFM) The patient suffering from this condition develops externally like a female At puberty breast development is normal and behaviour is female The reason for this is the following In the normal person testosterone is converted to an estrogen by an enzyme that is present also in individuals with TFM These persons may have high testosterone levels at puberty and this paradoxically leads to high estrogens! In spite of this the patients have male gonads and their chromosomal sex is male XY but they have no male secondary sex characteristics This and other clinical observations have shown that the early human fetus is female and only the incretion of androgens from an activated fetal testis produces a male individual If androgen is inactive even genetic (XY) males remain female as in TFM and medication with testosterone has no effect It is clear however that in the absence of ovaries there is no early development of uterus and vagina during fetal life These patients lack receptors for androgens Therefore it is believed that the *structural gene for this receptor is located on the X* chromosome but some observations that we have made in Malmo on members of a family with hemophilia in a boy (girl) with TFM are perhaps not consistent with this idea (8 10) Work on this family is in progress

The heredity of this condition is interesting in so far as the affected individuals who are always male are sterile and the disease can therefore only be propagated through true females who do not suffer any harm

Another possible instance of a refractory end or gan is also connected with fetal sexual differentia tion It is known that testosterone from the fe[illegible] testis induces development of male accesso[illegible] gans At the same time the Mullerian ducts th[illegible] developed in the female into oviduct and [illegible] inhibited in this development It has [illegible] that a special hormone from the testis is [illegible] for this effect The problem h[illegible]s b[illegible] [illegible] in vestigated by French authors (7) [illegible]

this process does not occur The reason may be defective formation of the anti Mullerian hormone The possibility that the cells of the duct are refractory has also been discussed Some facts indicate that this might be a genetic defect (2)

One of the most interesting examples of a refractory state against a hormone was first described by Hans Forssman in Uppsala This author studied hereditary diabetes insipidus It was well known that it may occur as an inherited dominant trait These patients respond very well to treatment with ADH This polypeptide is deficient Some of Forssman s patients did not respond to ADH and he was able to study this new disease in great detail also from the point of view of inheritance These patients with so called nephrogenic insipidus may well have an inherited defect in some part of the receptor mechanism (6 7)

One of the most intriguing questions in metabolism is connected with the mode of action of steroid hormones It has long been clear that anabolic steroids act on protein synthesis causing the build up of body substance The question how this works has recently been studied on a very suitable object namely the oviduct of the chick If the animal is treated with progesterone it has been found that the organ grows about 200 times very rapidly At the same time there is of course a change in protein synthesis and several authors have been able to demonstrate the accumulation of a biologically active messenger RNA that codes for ovalbumin When the hormone is withdrawn synthesis of this protein was found to become less than 1% During the hormonal treatment it is found that receptor proteins appear in the cytoplasm that seem to transport steroid hormones into the nuclear compartment where the receptor hormone binds to acceptor sites on the nuclear chromatin The last and final stage is activation of the process of protein formation by synthesis of specific RNA Several authors have studied the physico chemical characters of this receptor and it is clear that there is a quantitative specificity for the receptor effect in one organ This obviously directs the hormones to the cell nuclei where it is needed

We have reason to believe that similar mechanisms occur in other cells with other steroid hormones and it is very probable that TFM is the result of some such receptor defect

Several authors have studied the possibility that a defect in insulin binding may be the explanation of insulin resistance Already in 1973 Roth (13) studied this problem in mice In 1976 he continued this work on patients with insulin resistance and at the same time acanthosis nigricans From his study of six cases he concluded that there may be two groups of such patients one with a marked decrease in insulin binding to its receptor sites and another with the membrane receptor attacked by a circulating antibody All patients had severe insulin resistance and dermatological disease One of the patients had received insulin up to 48 000 IU/day All these patients and seven others reported in the literature were females One patient had a complete remission of the insulin resistance and simultaneous clearing of the acanthosis nigricans The younger patients also had lipoatrophy (12) The group with circulating antibodies had hypergammaglobulinemia and positive ANF etc The fact that insulin levels in the blood are high is easily explained as a compensatory hypersecretion

An important new development of this chapter of internal medicine has recently been published (5) It has long been known that patients with myasthenia gravis may respond rather poorly to the usual drugs Some of these patients have increased polyclonal gammaglobulin and it has been assumed that some of them have deficient function of the neuromuscular junction that is obviously a receptor for acetyl choline Convincing experiments have shown that the damage to this receptor is caused by a specific antibody that can be transferred to animals and then causes a myasthenic reaction Plasmapheresis has been shown to have excellent but transitory effects in some patients if it is performed on a large scale that allows substantial decrease in antibody titers This will be discussed in another paper

A new important group of patients suffering from an inherited disease of a specific receptor has been described in recent years It has long been a subject for much discussion among genetically minded physicians how the different so-called lipidoses were inherited A more or less general rule states that genetic mutations express themselves by more or less fundamental changes in the function of some polypeptide or protein This would mean that the apoproteins in the different mutations leading to the lipidoses should be structurally abnormal and different

Some years ago two American investigators started to analyze the mechanism behind hyper

cholesterolemia of Fredrickson type II or what we in Scandinavia not without reason call Muller Harbitz disease (3) It has long been known that persons suffering from this disease have a genetic trait that is inherited as dominant autosomal The frequency of the trait in different populations varies considerably In some parts of Sweden it is much more common obviously because there has been a clustering of families just like acute porphyria is much more common in Lappland Persons who have inherited the mutation in double dose, are extremely rare They suffer from very severe atherosclerosis and may die from cardiac infarction already as children American authors started to collect such rare instances of the disease They assumed that such persons did not have any normal gene that helps the heterozygotes to get along for some decades without too severe symptoms To be homozygote for a gene that expresses itself as a dominant trait is of course very serious

Fibroblasts from these severely afflicted persons were collected from all over the United States and for the last publication it had been possible to obtain cells from 43 patients from 11 different countries (3)

By using labelled protein in LDL it was possible to follow the metabolic pathway of the substance It was transported to the cells and normally entered the cell membrane on its way to the cytoplasm No LDL was found in the diseased cells to penetrate the cell membrane and this meant that the cholesterol was not taken up and processed by the body cells Also lymphocytes had the same defect in the cell receptors on the surface of the membrane It was found that two types of diseased receptors existed one with complete absence of receptor function another with impaired function Similar examples of inherited defects leading to different degrees of impairment are well known e g families with slight or severe hemophilia The degree is always true to type in a given family

In 42 different persons these two types were observed but one among the 43 investigated persons had another pattern LDL was bound correctly to the receptor site on the cell surface but did not enter the cytoplasm Obviously it was a third different and special mutation but leading to the same clinical picture as the other two mutations This shows how important it may be to repeat experiments many times before stating that findings have general application Interestingly this boy s parents were of two different types as regards LDL uptake One had a complete absence of receptor the other could not bring the LDL into the cytoplasm Both these mutated alleles existed in the boy s cells

How would these findings explain the fact that such persons develop massive hypercholesterolemia with cholesterol deposits in the arterial walls? Brown and Goldstein (3) explain this by the fact that cholesterol cannot be delivered to the cells and therefore stays in the circulation building up high blood levels that favor precipitation in certain tissues such as vessel walls and tendons These findings shed new light on the very interesting problem how we should interpret the mechanisms behind this interesting disease but it also gives a striking example of sick receptors

*Jan G Waldenstrom*

## REFERENCES


1 Albright F Burnett C H Smith P H & Parson W Pseudo hypoparathyroidism an example of Seabright Bantam syndrome Endocrinology 30 922–932 1942
2 Brook C G D et al Familial occurrence of persistent Mullerian structures in otherwise normal males Br Med J I 771–773 1973
3 Brown M S & Goldstein J L Familial hypercholesterolemia model for genetic receptor disease Harvey Lect 73 163–201 1977–78
4 Cuatrecasas P Insulin receptor interactions in adipose tissue cells direct measurement and properties Proc Natl Acad Sci 68 1264–1268 1971
5 Drachman D B Myasthenia gravis N Engl J Med 298 136–142 1978
6 Forssman H Two different mutations of the X chromosome causing diabetes insipidus Am J Hum Genet 7 21–27 1955
7 — The recognition of nephrogenic diabetes insipidus Acta Med Scand 197 1–6 1975
8 Holmberg L Genetic studies in a family with testicular feminization hemophilia A and colour blindness Clin Genet 3 253–257 1972
9 Josso N L Hormone antimullerienne une foeto protéine Arch Fr Pediatr 32 109–111 1975
10 Nilsson I M Bergman S Reitalu J & Waldenstrom J Haemophilia A in a girl with male sex-chromatin pattern Lancet 2 264–266 1959
11 O Malley B W Studies on the molecular mechanism of steroid hormone action Harvey Lect 72 53–90 1976–77
12 Pulini M et al Insulin resistance and acanthosis nigricans Report of a case with antibodies to insulin receptors Ann Intern Med 85 749–751 1976
13 Roth J Peptide hormone binding to receptors a review of direct studies in vitro Metabolism 22 1059–1073 1973

# BOOK REVIEW



*S Ohno Major Sex Determining Genes* In Monographs on Endocrinology vol 11 Edited by F Gross Labhard et al 140 pages Springer Verlag Berlin Heidelberg and New York 1979

The central theme of this book is that initially we are all females The male Y chromosome is in some way connected with the so-called HY antigen This according to the author is the testis organizing factor that starts proliferation of Leydig cells This produces testosterone and this hormone masculinizes the initially female structure of the foetal organism At the same time the Sertoli cells in the foetus produce an anti Mullerian factor probably of polypeptide nature that causes the involution of the Mullerian ducts It is interesting that the special hereditary syndrome called the persistent oviduct syndrome has been described in man This may either be caused by lack of or malformation of the "anti Mullerian hormone" or by a sick receptor for this hormone on the duct cells This important problem of sick receptors is treated in an editorial of this number of the journal

Medical technology has now reached the point when it is possible to determine the infinitely small amounts of testosterone present in foetal blood and organs at the gestation age around 60–90 days Circulating testosterone levels at this time and for some further two months approach the low normal levels in adult human males It is thus evident that the foetal testis is very active This is the period when the accessory sex organs become masculinized The editorial mentioned describes similar problems

This is in many ways a fascinating book even if the title seems very matter-of fact. It contains a wealth of facts some of which will become much discussed because they are so important But there is also a lively presentation of the biological philosophy adopted by one of the leading world authorities on sex determination and the biological basis for male chauvinism the author gives stimulating arguments in the battle about the "equality of the sexes" His ideas about sexology in general biology and in human history are also interesting

Above all for the biologists this is a goldmine even if certain chapters are highly specialized

*Jan G Waldenstrom*

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# Pseudohypoparathyroidism

*A 25 Year Delay in Diagnosis*

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ABSTRACT The diagnosis of pseudohypoparathyroidism is often not made until many years after the onset of symptoms and signs characteristic of the disease A case history presented here illustrates this and stresses the importance of determining the calcium concentration in plasma in all patients with attacks of sensory and motor phenomena

*Key words* pseudohypoparathyroidism hypoparathyroidism hypocalcaemia cyclic AMP

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Hypoparathyroidism (HP) not cuased by surgery of the thyroid or parathyroid glands is a rare disease (3 18) It occurs in two forms idiopathic hypoparathyroidism (IHP) and pseudohypoparathyroidism (PHP) IHP was first described in 1939 by Drake et al (7) In 1942 Albright et al (1) reported the characteristics of PHP

PHP is characterized by attacks of sensory and motor phenomena secondary to hypocalcaemia ectopic calcifications and developmental abnormalities of the skeleton The disease is diagnostically distinguished from IHP by the presence of a normal to increased concentration of parathyroid hormone (PTH) in serum concomitant with receptor resistance to PTH in the kidney and at times in bone tissue PHP is often diagnosed at a late stage and incidentally after several years of symptoms These have often been erroneously interpreted with subsequent irrational and ineffective treatment often with anticonvulsants (3 6 16) Such a case is reported here

## CASE REPORT

A 40-year-old man hospitalized for epilepsy An aunt (resident in Germany) has attacks which are treated with phenytoin The parents and an older sister are healthy The patient grew up in Hamburg under stressing conditions during the Second World War He suffered from meningoencephalitis at the age of 12 Serum calcium was not determined at that time

From the age of 14 the patient has had attacks of tingling sensations followed by seconds to minutes of cramps and reduced strength in the lower extremities followed by collapse He has never suffered from clonic cramps or loss of consciousness The attacks were provoked by mental or physical stress and occurred at intervals of months during the first years The frequency of attacks increased with time occurring several times a day immediately prior to hospitalization

The patient had been admitted to the Neurological Department of this hospital in 1954 and 1960 due to disturbance of gait The diagnosis was observation for schizophrenia In 1960 a S-calcium level of 7 5 mg/100 ml remained unnoticed In the same year he was admitted to the Psychiatric Department where the diagnoses of hysterical neurosis and psychoinfantilism were made

In 1958 and 1972 the patient was admitted to hospital for tonsillectomy and operation for hydrocele of the testes respectively S-calcium was not determined during these admissions He has been on continuous medication with phenytoin initiated by a German neurologist without improvement of the symptoms

In our department the patient was found to be mentally somewhat eccentric unusually courteous and meticulous but intellectually normal Periodically his gait was somewhat stiff but not atactic spastic or paretic No attacks occurred during hospitalization Epilepsy was considered unlikely from the physical examination and the EEG and phenytoin was therefore discontinued

Normal height (178 cm) and weight (66 kg) were found at physical examination The shape of his head was round with baldness of the forehead Chvostek s sign was positive There were increased interspaces between his teeth defects in the enamel as well as in the roots and several impacted teeth The laboratory tests repeatedly revealed a low S-calcium (min 1 29 mmol/l normal 2 29–2 67) and an increased S-phosphate (max 2 05 mmol/l normal 0 79–1 56) Alkaline phosphatase and S albumin levels

*Abbreviations* HP=hypoparathyroidism IHP=idiopathic HP PHP=pseudo HP PTH=parathyroid hormone c AMP=3 ,5 adenosine monophosphate 25 $OHD_3$= 25-hydroxycholecalciferol 1 25-$(OH)_2$ $D_3$=1 25-dihydroxycholecalciferol

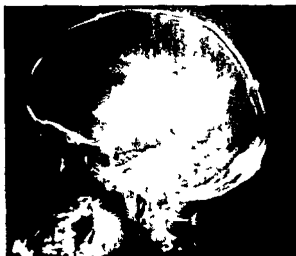


*Fig 1* X ray of the skull showing intracranial calcification of the basal ganglia choroid plexus and posterior fossa

were normal The concentration of PTH in serum was slightly increased (0 64 μg/l normal 0 22–0 50) whereas S thyroxin triiodothyronine resin test thyroid stimulating hormone S-creatinine and S-electrolytes were normal Calcifications of the basal ganglia the choroid plexus and posterior fossa were demonstrated by X ray (Fig 1) These calcifications could also be observed on the films of the cranium taken in 1954 when the patient was 16 years of age Slightly shortened metacarpal bones could be seen on the films of the hands (Fig 2) No osteitis fibrosa cystica or subcutaneous calcifications could be demonstrated Ophthalmological examination (including slit lamp examination) showed no metastatic calcifications

It is of interest that X rays of the hands of the patient s sister and mother also showed slight shortening of the metacarpal bones X rays of the skull and S-calcium levels were however normal These findings are compatible with the diagnosis of pseudo-pseudohypoparathyroidism in the sister and mother (19)

The patient was treated with Oleum calciferoli ultraconcentratum* initially 162 000 IU daily and with Calcium Sandoz* 2 g daily The dosage of vitamin D was reduced to 135 000 IU daily and the calcium supplement was discontinued after normalization of the S-calcium At that time the S PTH and S phosphate also became normal The levels of S calcium phosphate and PTH have remained normal since then The patient has been free from attacks and in good health

A modified Elsworth Howard test was carried out following normalization of the S-calcium phosphate and PTH levels (22 23) After injection of 300 USP units of bovine PTH (Lilly) for 5 min no cyclic 3 5 adenosine monophosphate (c AMP) could be demonstrated in the plasma or urine during the following two hours while injection of the same amount from the same batch to a patient with IHP brought about a pronounced rise in c AMP in the plasma and excretion of c AMP in the urine The diagnosis of PHP was thus verified in our patient

## DISCUSSION

PHP and IHP present in the main with the same symptomatology dominated by tetany tingling and convulsive attacks A reduction in height round head baldness intracranial calcifications shortened metacarpal bones and mental retardation occur more frequently in PHP than in IHP The concentration of PTH is normal or increased in cases of PHP but low in IHP (15 19 25) The differential diagnosis between PHP and IHP is made by measuring c AMP in plasma and urine after stimulation with bovine PTH (22 23 25) A very slight rise in the plasma concentration and excretion in the urine (less than twice the basal level) can be seen in PHP while in IHP a 3–10 fold increase occurs in the concentration in plasma and up to 100-fold in the excretion in urine (22 24)

The tissues normally sensitive to PTH are kidney bone and indirectly the bowel (5 19) In PHP there is a receptor resistance to PTH with no liberation of c AMP while in IHP there is normal receptor sensitivity (4 19 22) In some cases PHP may be accompanied by osteitis fibrosa cystica This is interpreted as a condition in which receptor sensitivity is retained in bone tissue but lost in kidney tissue (2 9 14 19) A lowered c AMP response to exogenous PTH is observed in conditions with high concentrations of PTH in the plasma as well as in conditions with a high S creatinine (15 25) At the time of the PTH stimulation test our patient had normal plasma PTH and S-creatinine




*Fig 2* X ray of the hands showing slight shortening of the 3rd 4th and 5th metacarpal bones

levels In patients with PHP the increased plasma PTH levels are presumably secondary to a defect in the 1α hydroxylation of 25 hydroxycholecalciferol (25-OHD$_3$) in the kidneys with subsequent reduction in the intestinal calcium absorption and hypocalcaemia (11 19 20) The serum concentration of 1 25-dihydroxycholecalciferol (1 25-(OH)$_2$ D$_3$) is low while 25 OHD$_3$ is normal in cases of PHP The intestinal calcium absorption is often increased after administration of 1 25(OH)$_2$ D$_3$ concomitant with a normalization of the serum PTH and serum calcium levels (5 8 20) although one case has been described in whom this effect of 1 25(OH)$_2$ D$_3$ could not be demonstrated (13) Recent evidence indicates that c AMP must be presumed to play an important role in the stimulation of 1 25 (OH)$_2$ D$_3$ formation induced by PTH (11) In normal persons and in patients with IHP the formation of c AMP after stimulation with PTH takes place mainly in the kidneys (12 22) The half life of exogenous PTH in plasma is 4–6 min whereas that of c AMP is 14 min (22)

Genetically PHP is a sex linked dominant hereditary disease with variable penetration (17) The long period between onset of symptoms and diagnosis is unfortunately a frequent phenomenon in cases of PHP and IHP (3 6 10 16 21) Periods of up to 40 years have been reported A contributory cause is the rareness of the disease which however does not excuse a delay of several years in the diagnosis of the condition in which the majority of patients could be kept free from symptoms by means of a simple treatment Determination of the S-calcium levels should be obligatory in the initial screening procedures in all patients with attacks of sensory and motor phenomena

## REFERENCES


1 Albright F Burnett C H Smith P H & Parson W Pseudohypoparathyroidism an example of "Seabright Bantam Syndrome" Endocrinology 30 922 1942
2 Bell N H Gerard E S & Bartter F C Pseudohypoparathyroidism with osteitis fibrosa cystica and impaired absorption of calcium J Clin Endocrinol 23 759 1963
3 Bronsky D Kushner D S Dubin A & Snapper I Idiopathic hypoparathyroidism and pseudohypoparathyroidism case reports and review of the literature Medicine 37 317 1958
4 Chase L R Melson G L & Aurbach G D Pseudohypoparathyroidism Defective excretion of 3 5 -AMP in response to parathyroid hormone J Clin Invest 48 1832 1969
5 DeLuca, H F Recent advances in our understanding of the vitamin D endocrine system J Lab Clin Med 87 7 1976
6 Dimich A Bedrossian P B & Wallach S Hypoparathyroidism Clinical observations in 34 patients Arch Intern Med 120 449 1967
7 Drake T G Albright F Bauer W & Castleman B Chronic idiopathic hypoparathyroidism report of six cases with autopsy findings in one Ann Intern Med 12 1751 1939
8 Drezner M K Neelon F A Haussler M McPherson H T & Lebovitz H E 1 25 Dihydroxycholecalciferol deficiency The probable cause of hypocalcemia and metabolic bone disease in pseudohypoparathyroidism J Clin Endocrinol 42 621 1976
9 Frame B Hanson C A Frost H M Block M & Arnstein A R Renal resistance to parathyroid hormone with osteitis fibrosa "Pseudohypohyperparathyroidism" Am J Med 57 311 1972
10 Gsell O Chronische idiopathische Tetanie (mit Psoriasis) (hypoparathyreoider Kretinismus) Dtsch Med Wochenschr 35 1117 1950
11 Horiuchi N Suda, T Takahashi H Shimazawa, E & Ogata E In vivo evidence for the intermediary role of 3 5 cyclic AMP in parathyroid hormone induced stimulation of 1α 25 dihydroxyvitamin D$_3$ synthesis in rats Endocrinology 101 696 1977
12 Kaminsky N I Broadus A E Hardman J G Jones Jr D J Ball J H Sutherland E W & Liddle G W Effects of parathyroid hormone on plasma and urinary adenosine 3 5 monophosphate in man J Clin Invest 49 2387 1970
13 Kind H P Prader A DeLuca H F & Gugler E 1,25-Dihydroxycholecalciferol in hypoparathyroidism and pseudohypoparathyroidism Lancet I 1145 1975
14 Kolb F O & Steinbach H L Pseudohypoparathyroidism with secondary hyperparathyroidism and osteitis fibrosa. J Clin Endocrinol 22 59 1962
15 Lewin I G Papapoulos S E Tomlinson S Hendy G N & O Riordan J L H Renal adenyl cyclase responsiveness and hyperparathyroidism of chronic renal failure Clin Sci Mol Med 54 27 P 1978
16 — Studies of hypoparathyroidism and pseudohypoparathyroidism Q J Med 47 533 1978
17 Mann J B Alterman S & Hills A G Albright s hereditary osteodystrophy comprising pseudohypoparathyroidism and pseudo-pseudohypoparathyroidism. With a report of two cases representing the complete syndrome occurring in successive generations Ann Intern Med 56 315 1962
18 Philipson B Angelin B Christensson T Einarsson K & Leyd B Hypocalcaemia with zonular cataract due to idiopathic hypoparathyroidism Acta Med Scand 203 223 1978
19 Potts J T Jr Pseudohypoparathyroidism In The metabolic basis of inherited disease 4th ed (ed J B

Stanbury J B Wyngaarden and D S Fredrickson) pp 1350–1365 McGraw Hill New York 1978
20 Sinha T K DeLuca H F & Bell N H Evidence for a defect in the formation of 1α 25-dihydroxyvitamin D in pseudohypoparathyroidism Metabolism 26 731 1977
21 Steinberg H & Waldron B R Idiopathic hypoparathyroidism An analysis of fifty two cases including the report of a new case Medicine 31 133 1952
22 Tomlinson S Barling P M Albano J D M Brown B L & O Riordan J L H The effects of exogenous parathyroid hormone on plasma and urinary adenosine 3 5 cyclic monophosphate in man Clin Sci Mol Med 47 481 1974
23 Tomlinson S Hendy G N & O Riordan J L H A simplified assessment of response to parathyroid hormone in hypoparathyroid patients Lancet i 62 1976
24 Tomlinson S Hendy G N Pemberton D M & O Riordan J L H Reversible resistance to the renal action of parathyroid hormone in man Clin Sci Mol Med 51 59 1976
25 Woodhouse N J Y Hypocalcaemia and hypoparathyroidism Clin Endocrinol Metab III 323 1974

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# Localization of Aldosterone-Producing Tumours in Primary Aldosteronism by Adrenal and Renal Vein Catheterization

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**ABSTRACT** Regional venous plasma aldosterone concentrations were determined and assessed against concurrent arterial levels in 16 patients with primary aldosteronism The results obtained by sampling from the left adrenal vein or the left renal vein allowed correct side prediction of the presupposed adenoma in each patient. The problems caused by intermittent secretion of aldosterone by the tumour and the importance of correct positioning of the catheter are emphasized Repeated sampling and *continuing reference to systemic arterial aldosterone* levels proved valuable

*Key words* adrenal veins aldosterone producing adenomas plasma aldosterone primary aldosteronism renal veins

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Preoperative localization of an aldosterone producing adenoma in primary aldosteronism (Conn s syndrome) simplifies the surgical procedure (4 6) Selective adrenal venography has proven the most sensitive radiologic method in detection of these tumours (4 5 13 22) However severe extravasation of contrast material (2 13 18) or even necrosis of one or both adrenal glands (1 8 23) have been reported after venography

Sampling of blood from the venous efflux of endocine glands is a well known principle for localization of hormone producing tumours The development of sensitive and accurate assays for plasma aldosterone has encouraged the use of this principle in primary aldosteronism (3 5 8 11 17 18 20) We present our experience with plasma aldosterone determinations in samples obtained by venous catheterization in patients with aldosterone producing tumours

## PATIENTS

Sixteen patients with primary aldosteronism were studied (Table I) The diagnosis was based on the presence of hypertension hypokalemia low plasma renin concentration and high tetrahydroaldosterone excretion rate In all cases plasma renin concentration was below 15 mIU/ml and the aldosterone production was autonomous as demonstrated by a fludrocortisone suppression test (15)

Diuretic therapy including spironolactone was withdrawn in all patients at least 14 days before the study Three patients (nos 12–14) were treated with methyldopa hydralazine or prazosin at the time of investigation The patients took a normal diet without restriction in sodium intake

## METHODS

### *Biochemical procedures*

Plasma renin concentration (10) plasma angiotensin II (14) and urinary tetrahydroaldosterone (19) were measured by previously described methods

Plasma aldosterone concentration (PAC) was measured by a modification of the radioimmunological method of Mayes et al (16) Antialdosterone serum (Ewe) was obtained from Research Plus Denville New Jersey USA (Prod no 1-0196) and separation of free and antibody bound aldosterone was performed by gel centrifugation (10) The between assay coefficient of variation was 13% as calculated from 18 duplicate determinations at levels ranging from 5 to 70 ng/100 ml In normal supine subjects on a free diet peripheral PAC ranged from 3 to 18 ng/100 ml No interference from amidotrizoate (Urografin®) was seen

Cortisol in plasma was measured by means of a commercially available kit (Diagnostic Products Cooperation

Table I *Clinical data and laboratory findings*

*PRC*=plasma renin concentration PA II=plasma angiotensin II concentration UTH aldo=urinary tetrahydroaldosterone excretion rate

| Pat no | Sex | Age (y) | BP (mmHg) | Plasma potassium (mEq/l) | Supine PRC (mIU/l) | PA II (pg/ml) | UTH aldo (μg/24 h) |
|---|---|---|---|---|---|---|---|
| 1 | ♀ | 56 | 177/111 | 2 1 | 9 | – | 264 |
| 2 | ♀ | 55 | 200/115 | 2 2 | 10 | – | 195 |
| 3 | ♀ | 33 | 190/120 | 2 1 | 3 | – | 92 |
| 4 | ♂ | 11 | 168/122 | 2 8 | 1 | – | 103 |
| 5 | ♀ | 51 | 165/115 | 2 1 | 5 | – | 234 |
| 6 | ♀ | 39 | 160/110 | 2 7 | 7 | – | 52 |
| 7 | ♀ | 52 | 210/117 | 2 5 | 10 | 8 | 68 |
| 8 | ♂ | 57 | 180/110 | 3 0 | 3 | <3 | 99 |
| 9 | ♀ | 47 | 165/110 | 2 1 | 4 | <3 | 287 |
| 10 | ♂ | 23 | 175/120 | 3 1 | 11 | <3 | 62 |
| 11 | ♀ | 56 | 200/120 | 2 6 | 8 | – | 90 |
| 12 | ♀ | 55 | 270/150 | 2 3 | 7 | – | 84 |
| 13 | ♀ | 24 | 190/115 | 2 8 | 5 | <3 | 120 |
| 14 | ♀ | 47 | 200/120 | 1 8 | 7 | <3 | 144 |
| 15 | ♂ | 48 | 186/117 | 3 2 | 11 | 5 | 161 |
| 16 | ♂ | 49 | 177/112 | 3 3 | 7 | <3 | 96 |
| Normal range | | | | 3 6–4 4 | 6–59 | <3–10 | 11–59 |

Los Angeles California USA) The between assay variation was 6% and peripheral venous plasma levels in normal subjects measured between 8 and 11 a m ranged from 5 to 26 μg/100 ml

*Catheterization procedure*

If necessary the patients were premedicated with 5–10 mg diazepam A venous cutdown in the cubital region was performed in 13 cases A Vaniflex® catheter with two side holes 1 cm from the tip was introduced and the catheter was manipulated by means of a Pilotip® guide and Rotoflector® handle (USCI International Inc New Yersey USA) In five cases including two cases mentioned above one or two catheters were inserted via the femoral veins using the Seldinger technique A catheter preshaped for renal vein catheterization (Angiodan no 0870 G 252 Surgimed Olstykke Denmark) or for adrenal vein catheterization (type BP 7 MK 1 A William Cook Europe Søborg Denmark) was used for the femoral technique A small polyethylene catheter was inserted into the brachial or femoral artery

As a routine samples were drawn from the left adrenal vein three positions in the left renal vein the right renal vein and from the right side of the inferior caval vein at various levels above the right renal vein Finally catheterization of the right adrenal vein was attempted Anatomical details were visualized by X ray fluoroscopy after rapid injection of 10–20 ml Urografin® 76% into the left renal vein In a few cases 0 5–2 ml Urografin® 45% was injected slowly into the adrenal vein but no attempt was made to perform selective adrenal venography Oxygen saturation in blood samples from adrenal or renal veins was determined by a Kipp Hemoreflector Along with the venous sampling lasting for 1–2 hours 3–6 arterial samples for reference were collected at appropriate intervals

The blood samples were drawn into dry syringes and transferred to tubes with disodium EDTA (15 mg/10 ml blood) A maximum volume of 300 ml blood was removed during the total catheterization procedure and replaced with isotonic saline used for flushing the catheters In adrenal vein sampling the blood was allowed to drip freely or was drawn by application of minimal suction at a rate of 1–2 ml/min Plasma was separated within 90 min and stored at −18°C until analysis

*Calculations*

The PAC of each regional venous sample was compared with the simultaneous arterial PAC or—if this was not

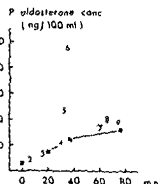



*Fig 1* Arterial (■–■) and regional venous (encircled numerals) plasma aldosterone concentrations in samples obtained during catheterization from patient 2 with a right sided adrenal adenoma 1 – 3 7 9=left renal vein 4=right renal vein 5 – 6 = inferior caval vein 8 = left adrenal vein Only the venous values 5 and 6 differ significantly from the concurrent arterial values

measured at the actual time of venous sampling—an estimate of the arterial PAC obtained by interpolation (Fig 1) Based upon the knowledge of the coefficient of variation the significance of a veno-arterial difference was calculated from

$$t = \frac{C_v - C_a}{0.13\sqrt{C_v^2 + C_a^2}}$$

$C_v$ and $C_a$ represent the regional venous and systemic arterial PAC respectively A $t$ value higher than 2 indicated a significant difference between the regional venous and the concurrent arterial concentration ($p<0.05$) The significance of differences in cortisol values was calculated in an analogous way substituting the coefficient of variation with the value found for the cortisol kit

## RESULTS

No complications were observed during or after the studies *Occasionally slight flank pain occurred* during manipulation of the catheter and a vasovagal attack developed in two patients at the end of the catheterization Catheterization of the left adrenal vein was successful in 12 patients whereas the right adrenal vein was located in only 4 In one patient the manipulation of the catheter was particularly difficult and cannulation of the left renal vein was omitted

Table II shows PAC in the samples obtained In 8 patients the arterial PAC fluctuated considerably during the study Thirteen patients had a consistently high arterial PAC Normal arterial PAC was seen in some samples of two patients and throughout the study in one patient

In nine patients (nos 7–10 12–16) very high levels of PAC were measured in samples from the left adrenal vein In patients 4 and 6 as well as in eight of the patients mentioned above (nos 7–10 12–14 16) significantly increased PAC was found in samples from the left renal vein With the proviso that an adrenal adenoma would be unilateral a left sided lesion was predicted in these 11 patients In patient 6 PAC in the left adrenal vein equalled the arterial concentration

In five patients (nos 1–3 5 11) PAC in samples from the left adrenal vein (two patients) and from the left renal vein were equal to the arterial concentration Thus no aldosterone efflux from the left gland was demonstrated and a right sided lesion was predicted in these patients This prediction was further supported by the actual demonstration of increased values in samples from three patients (nos 2 3 5) obtained from locations on the right side of the inferior caval vein

In samples from the right renal vein PAC equalled the arterial concentration in all cases except one (no 12) *in whom the arterial PAC though low* fluctuated considerably In samples from the right side of the inferior caval vein increased values in relation to the arterial levels were seen in 9 patients High values were seen in patients with right as well as left sided lesions In one patient (no 5) concentrations comparable to those found in adrenal veins draining an adenoma were measured In four patients the right adrenal vein was cannulated Aldosterone levels equal to the arterial levels were seen in two and moderate increases in the two others The left/right ratio between the levels in the adrenal veins was 20–251 in these cases in whom a left sided adenoma was predicted

*Plasma cortisol was measured in the samples* from 5 patients (nos 12–16) and the values are given in Table III The arterial plasma cortisol was within the normal range in all patients Increased venous plasma cortisol in relation to the arterial levels was found in ten adrenal venous samples No difference in veno-arterial plasma cortisol was disclosed in three adrenal venous samples In the samples from the left renal vein and the inferior caval vein a significant veno-arterial difference was demonstrated in only one sample (pat 13)

Oxygen saturation in left adrenal venous blood was measured in 9 patients Values of 66 and 78% respectively were found in two cases and 85–91% *in seven In the left renal vein peripherally to the* orifice of the left adrenal vein the oxygen saturation ranged from 82 to 89%

### *Surgery and subsequent course*

*A lateral posterior approach* was applied in 14 cases and a transabdominal procedure with exposure of both adrenals in 2 A single adenoma with a maximum diameter of 8–35 mm was found in all patients 11 adenomas measured 15 mm or less As shown in Table II the prediction of localization was in accordance with the finding at surgery in all patients In two cases the adenoma could not be identified with certainty before the adrenal gland was sectioned and in several cases the small adenoma could be felt and seen only after extensive dissection Unilateral adrenalectomy was carried out in 15 patients and resection of the adrenal gland in one patient

Table II *Plasma aldosterone concentration (ng/100 ml) in venous and arterial blood samples obtained durin catheterization*

| Pat. no | Left renal vein[a] P | I | C | Left adrenal vein | | Right renal vein | Inferior caval vein | | | | Right adrenal vein |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 91 | 93 | 75 | – | | 150 | 125 | 182 | | | – |
| 2 | 8<br>34 | 14 | 7<br>38 | 41 | | 22 | 46* | 95* | | | – |
| 3 | 26<br>25 | | 34<br>26 | – | | 39 | 29 | 98 | | | – |
| 4 | 46<br>43 | 201* | 51<br>41 | – | | 45 | 50 | 67 | 74 | 78 | – |
| 5 | 60 | 54 | 60 | 68 | | 61 | 194 | 1 455 | 765* | | – |
| 6 | 30 | 88* | 37 | 38 | | 27 | | – | | | – |
| 7 | 33 | 43 | 26 | 450 | | 24 | 21 | 26 | 27 | | – |
| 8 | 47 | 49 | 80* | 1 700 | 7 400 | 46 | 37 | 41 | 32 | | – |
| 9 | 210* | 242* | 120 | 8 800* | | 103 | 114 | 92 | 170 | | – |
| 10 | <br>112* | 35<br>47 | 47 | 604 | | 27 | 43 | 45 | 44 | | 31 |
| 11 | 54 | 56 | 52 | – | | 56 | 61 | 66 | | | – |
| 12 | 9<br>18* | 7 | 8 | 6 050 | 6 900* | 19 | 11 | 36 | 9 | | – |
| 13 | 27 | 242* | 23 | 4 600* | 8 050 | 31 | 41 | 27 | 27 | | 32 |
| 14 | 39 | 93* | 172 | 3 500* | 9 700 | 41 | 75 | 28 | | | 126 |
| 15 | – | – | – | 96* | 997* | 34 | 30 | 28 | 43 | | – |
| 16 | 23 | 39 | 37* | 1 660* | 1 350 | 20 | 26 | 36 | | | 28 |

[a] Catheter position P = peripheral I = intermediate C = central
* Venous PAC significantly different from the arterial level ($p<0.05$)

Postoperatively plasma renin concentration urinary excretion rate of tetrahydroaldosterone and plasma potassium concentration normalized and the blood pressure became normal or decreased in all patients

## DISCUSSION

The technique applied in the present study proved valuable for planning the surgical procedure in that the correct side was predicted in all of our 16 consecutive patients undergoing adrenal surgery

Ideally bilateral adrenal vein samples should be collected (11 17 18) However the adrenal veins may be difficult to cannulate especially the short right adrenal vein Assuming the presence of a solitary adenoma valid conclusions can be drawn from the results of sampling from the left side only either from the adrenal or the renal vein as shown in the present study as well as in earlier reports (9 20) In fact the adenomas are nearly always tary In the present series the presence of a adenoma in the remaining gland was ruled out by the postoperative normalization of the biochemica parameters and amelioration of hypertension In reports concerning 84 surgically treated patients with aldosterone producing adenomas (4 5 9 11 17 18 20) only one patient had bilateral adenomas This low prevalence of bilateral adenomas justifies the present approach

Prior to catheterization it is important to differentiate between adrenocortical hyperplasia and adenoma The currently applied methods for this discrimination have recently been reviewed (7) In the present study the presence of an aldosterone producing adenoma was predicted from the biochemical profile including the autonomy of aldosterone production (15) Thus the pur

| Artery | | | | | | Localization of adenoma | |
|---|---|---|---|---|---|---|---|
| | | | | | | Predictive | Found at *surgery* |
| 68 | 119 | 215 | | | | Right | Right |
| 6 | 15 | 25 | 32 | | | Right | Right |
| 28 | 38 | 31 | 27 | 25 | | Right | Right |
| 54 | 52 | 54 | 54 | 43 | | Left | Left |
| 74 | 65 | 96 | 108 | | | Right | Right |
| 36 | 35 | 35 | 34 | 45 | | Left | Left |
| 23 | 22 | 20 | 17 | 20 | 19 | Left | Left |
| 36 | 43 | 40 | 27 | | | Left | Left |
| 68 | 108 | 89 | 107 | | | Left | Left |
| 38 | 31 | 32 | 42 | 40 | | Left | Left |
| 51 | 52 | 61 | 63 | | | Right | Right |
| 11 | 15 | 6 | 5 | 8 | 13 | Left | Left |
| 26 | 29 | 24 | | | | Left | Left |
| 28 | 36 | 50 | 50 | | | Left | Left |
| 58 | 23 | 24 | | | | Left | Left |
| 25 | 16 | 16 | | | | Left | Left |

pose of catheterization was solely to localize the adenoma

PAC may fluctuate in primary aldosteronism (24) presumably as a result of intermittent secretion Since multiple samplings in the venous system can *not be performed simultaneously PAC in the venous* samples from different regions may differ solely as a result of intermittent secretion This difficulty was overcome in the present study by strict reference to systemic arterial PAC In this way samples from veins draining aldosterone producing tissue could be identified Without correction for arterial PAC the interpretation of the results would be difficult in several cases e g nos 1 and 2

Since there is no influx of adrenal venous blood to the right renal vein no veno arterial difference in PAC is anticipated in samples from this location This was confirmed in our study and thus a check on the interpolation procedure as well as the precision of the aldosterone assay was obtained

In patient 6 PAC in the left vein was equal to the arterial level whereas a sample from the left renal vein identified the left sided adenoma This phenomenon may again be due to intermittent secretion of aldosterone Another point is that the left adrenal vein has tributaries from the inferior phrenic vein or renal capsular veins (22) and the blood actually sampled may well be from an extraadrenal source

The position of the catheter is obviously very important Owing to the reported complications we omitted a proper adrenal venography To ensure the correct catheter position only a very small amount of contrast material was injected in the left adrenal vein and a slender vein was visualized (13) Due to the high relative adrenal blood flow (12) a high venous oxygen saturation is expected Values of the same order of magnitude as in samples from the renal veins were found in the majority of samples from the left adrenal veins However low

Table III *Plasma cortisol concentration (μg/100 ml) in venous and arterial samples obtained during catheterization in 5 patients*

| Pat no | Left renal vein | | | Left adrenal vein | | Right renal vein | Inferior caval vein | | | Right adrenal vein | Artery | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | P | I | C | | | | | | | | | | | | | |
| 12 | 7 8 | 8 | 8 | 433 | 527 | 9 | 10 | 10 | 10 | – | 12 | 10 | 9 | 11 | 14 | 24 |
| 13 | 13 | 33 | 15 | 32 | 34 | 13 | 13 | 13 | 14 | 16 | 19 | 17 | 15 | | | |
| 14 | 5 | 5 | 7 | 29* | 564 | 5 | 6 | 7 | | 16* | 6 | 5 | 6 | 6 | | |
| | – | – | – | 10 | 15 | 7 | 7 | 7 | 7 | – | 18 | 10 | 6 | | | |
| 16 | 19 | 19 | 18 | 436 | 158 | 18 | 15 | 15 | | 14 | 25 | 25 | 17 | | | |

Abbreviations for catheter position and statistical symbols as in Table II

Act

oxygen saturation i e levels comparable to those of the inferior caval vein was also seen Thus measurement of oxygen saturation did not allow a confident discrimination between adrenal and renal venous blood on the left side or between adrenal and peripheral blood

*Adjuvant determination of plasma cortisol concentration* in the samples has also been advocated for the identification of samples containing adrenal blood (17 20) Significant veno arterial cortisol differences identified the presence of blood from steroid producing tissue in the majority of adrenal vein samples but adrenal venous levels equal to the arterial levels were also seen in samples with very high PAC The periodical secretion of cortisol may explain this phenomenon (21) Accordingly cortisol determinations may confirm the actual presence of adrenal vein blood in a sample but the absence of a significant veno arterial difference does not exclude correct sampling of adrenal venous blood

In samples from the right side of the inferior caval vein the efflux from right sided adenomas was demonstrated in 3 cases However in two patients positive evidence of right sided tumour was not obtained and increased PAC in samples from the inferior caval vein was also demonstrated in patients with left sided adenomas

PAC in veins draining tumour bearing glands ranged from 96 to 9700 ng/100 ml values corresponding well to those reported by others (9 11 17 18 20) Large sample to sample fluctuations were seen in the individual case In seven cases adrenal blood was obtained from the non tumour gland In 5 cases PAC was equal to the arterial level and slightly elevated values were found in two suggesting that the contralateral gland had not been completely suppressed

In patients assumed to harbour an aldosterone producing adenoma determination of PAC in the left adrenal vein (or the left renal vein) as assessed by comparison with the contemporary arterial levels proved sufficient to localize the adenoma Emphasis should be given to the problem of intermittent secretion of aldosterone and to the possibility of sampling from the inferior phrenic vein Multiple sampling is recommended at least two adrenal vein samples or two series from the renal vein should be obtained at an interval of 10–15 min Repositioning of the catheter in the left adrenal vein can be important in order to obtain a proper adrenal venous blood sample


## ACKNOWLEDGEMENT

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## REFERENCES


1 Bayliss R I S Edwards O M & Starer F Complications of adrenal venography Br J Radiol 43 ..3 1970
2 Bookstein J J Conn J & Reuter S R Intra adrenal hemorrhage as a complication of adrenal venography in primary aldosteronism Radiology 9( 778 1968
3 Bucht H Bergstrom J Lindholmer B Wijnbladh H & Hokfelt B Catheterization of the left adrenal vein for contrast injection and steroid analysis in case of Conn s syndrome Acta Med Scand 176 233 1964
4 Conn J W Rovner D R Cohen E L Bookstein J J Cerny J C & Lucas C P Preoperative diagnosis of primary aldosteronism including a comparison of operative findings and preoperative tumour localization by adrenal phlebography Arch Intern Med 123 113 1969
5 Davidson J K Morley P Hurley G D & Holford N G H Adrenal venography and ultrasound in the investigation of the adrenal gland an analysis of 58 cases Br J Radiol 48 435 1975
6 Egdahl R H Surgery of the adrenal gland N Engl J Med 278 939 1968
7 Ferriss J B Beevers D G Brown J J Fraser R Lever A F Padfield P L & Robertson J I S Low renin ( primary ) hyperaldosteronism Differential diagnosis and distinction of sub-groups with in the syndrome Am Heart J 95 641 1978
8 Fischer C E Turner F A & Horton R Remission of primary hyperaldosteronism after adrenal venography N Engl J Med 285 334 1971
9 Fukuchi S Takanouchi T Nakajima K Watanabe H & Sugita A Location of aldosterone producing adenomas by the determination of plasma aldosterone in adrenal vein or renal vein blood Clin Sci Mol Med 49 187 1975
10 Giese J Jorgensen M Nielsen M D Lund J O & Munck O Plasma renin concentration measured by use of radioimmunoassay for angiotensin I Scand J Clin Lab Invest 26 355 1970
11 Horton R & Finck E Diagnosis and localization in primary aldosteronism Ann Intern Med 76 885 1972
12 Hume D C Bell C C & Bartter F Direct measurement of adrenal secretion during operative trauma and convalescence Surgery 52 174 1962
13 Kahn P C Kelleher M D Egdahl R H & Melby J C Adrenal arteriography and venography in primary aldosteronism Radiology 101 71 1971
14 Kappelgaard A M Damkjær Nielsen M & Giese J Measurement of angiotensin II in human plasma Technical modification and practical experience Clin Chim Acta 67 299 1976
15 Lund J O & Damkjær Nielsen M Fludrocortisone suppression test in normal subjects in patients with essential hypertension and in patients with various

forms of aldosteron sm Acta Endocrınol (Kbh) 93 100 1980

16 Mayes D Furuyama S Kem D C & Nugent C A A rad o mmunoassay for plasma aldosterone J Clın Endocrınol Metab 30 682 19 0

17 Melby J C Spark R F Dale S L Egdahl R H & Kahn P C D agnos s and local zat on of aldosterone produc ng adenomas by adrenal ve n ca heterızat on N Engl J Med 277 1050 1967

18 N col s G L M tty H A Modl nger R S & Gabrılove J L Percutaneous adrenal venography Ann Intern Med 76 899 1972

19 N elsen M D Lund J O & Munck O Urınary excret on of tetrahydroaldosterone n normal subjects and ın pat ents w th adrenal nsuff c ency and Conn s syndrome Acta Endocrınol (Kbh) 71 498 197?

?0 Scoggıns B A Odd e C J Hare W S C & Coghlan J P Preoperat ve la eral sat on of aldosterone p oduc ng tumours n prımary aldosteronısm Ann Intern Med 76 891 197?

21 Spark R F Ke tyle W R & E senberg H Cor t sol dynam cs n the adrenal venous effluent J Clın Endocrınol Metab 39 305 1974

?? Sutton D The rad olog cal dıagnos s of adrenal tumours Br J Rad ol 48 237 1975

23 Taylor H C & Sachs C R Prımary aldosteron sm Rem ss on and de elopment of adrenal nsuff c ency after adrenal venography Ann Intern Med 85 07 1976

24 Vetter H Berger M Armbruster H S egenthaler W Wern ng C & Vetter W Ep sod c secret on of aldosterone ın prımary aldosteron sm relatıonsh p to cort sol Clın Endocrınol 3 41 1974

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Acta Med Scand 207 353–357 1980

# Plasma ACTH in Patients with Bronchogenic Carcinoma

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**ABSTRACT Immunoreactive ACTH before and five hours after administration of 2 mg of dexamethasone was determined in patients examined in hospital for abnormalities on chest X ray Thirty patients had primary bronchial cancer, 15 had other lung conditions mostly inflammatory infiltrations The mean total ACTH and ACTH after dexamethasone suppression were significantly higher in the patients with bronchial cancer than in patients with other lung lesions or healthy controls ACTH in plasma after administration of glucocorticoids was predominantly big ACTH Several of the cancer patients had however, ACTH levels within the same range as the other subjects These results indicate that plasma ACTH determinations are of limited value as tumour markers for the detection of lung cancer although it is still possible that repeated sampling in patients with elevated ACTH levels may be of value when monitoring the therapy**

*Key words* bronchial cancer plasma ACTH plasma cortisol

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ACTH like material produced by non-endocrine neoplasms is one cause of Cushing s syndrome (12) Bronchial tumours are the most common constituting about 50% of the cases However when investigating numerous patients with bronchial cancer the incidence of Cushing s syndrome is low (18) The synthesis in and release of ACTH from such tumours is a more common finding than previously known Significant amounts of ACTH in bronchial tumours have also been found in patients without Cushing s syndrome (19) Gewirtz and Yalow (4) and Yalow and Berson (29) reported that ACTH in tumour extracts was predominantly big ACTH while 1–39 ACTH dominated in pituitary extracts

Big immunoreactive ACTH which has a molecular weight of at least 20 000 according to gel filtration is believed to be a prohormonal form of ACTH (4) This prohormone lacks significant biologic activity and hence production of big ACTH will not necessarily cause Cushing s syndrome If this precursor hormone is released into the blood preferentially from bronchial tumours it could serve as a tumour marker Elevated immunoreactive ACTH levels were indeed found in 21 out of 24 patients with untreated bronchial cancer and in 14 out of 36 patients with chronic obstructive pulmonary disease (1) A higher frequency of ACTH levels within the normal range was however found in subsequent studies with larger series of patients (27 28)

The aim of the present study was to evaluate the use of plasma immunoreactive ACTH as a marker for bronchogenic carcinoma In accordance with Gewirtz and Yalow (4) we observed that the high molecular weight form of ACTH was still identifiable in blood when 1–39 ACTH was suppressed by glucocorticoids In order to decrease the amount and variation of pituitary ACTH we have determined ACTH after dexamethasone administration and collected the blood samples in the afternoon

## SUBJECTS AND METHODS

The patient series comprised 45 patients referred to hospital for evaluation of pulmonary infiltrations evident on X-ray They were in good general condition and had no clinical evidence of Cushing s syndrome Thirty patients 20 males and 10 females age range 38–73 years who had primary bronchogenic carcinoma constituted the cancer group The diagnosis was verified by pathoanatomic examination or cytology The distribution according to tumour type was 17 squamous cell carcinomas nine oat cell carcinomas three adenocarcinomas and one small cell (undifferentiated) carcinoma All these patients were smokers except one patient with adenocarcinoma Fifteen patients seven males and eight females age range 35–73 years who had lung lesions other than bronchial carcinoma comprised the lung lesion group Nine of them had inflammatory infiltrations two metastases from renal carcinomas one sarcoidosis one pulmonary arteritis one

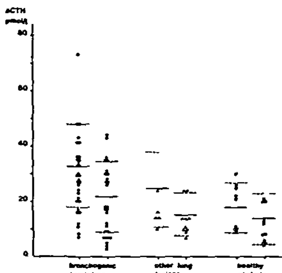



*Fig 1* Plasma ACTH levels at 3 p m in patients with bronchogenic carcinoma patients with various lung lesions and healthy controls The right column in each group shows ACTH levels at 5 hours after 2 mg oral dexamethasone —=Mean = S D

Hodgkin s disease and one bronchiectases Ten patients in this group were smokers Four patients were not included in either group two with bronchial cancer and additional malignant tumours and two on glucocorticoid medication The control group comprised 13 healthy volunteers two males and 11 females age range 21–60 years

Venous blood samples for ACTH and cortisol determinations were collected at 3 p m on two consecutive days n the second day the subjects received 2 mg dexamethasone orally at 10 a m. Blood samples were collected in siliconized and heparinized glass tubes centrifuged immediately and kept frozen below −20°C until assay

ACTH was measured by radioimmunoassay directly in plasma The properties of the antiserum indicated antigenic determinants mainly in the N-terminal part of the ACTH molecule C-terminal fragments and biologically inactive N terminal fragments lacking the first three amino acids did not cross-react The cross reaction of α MSA was 5% and negligible to β-MSH β-lipotropine and arginine vasopressin Synthetic human ACTH provided by Ciba-Geigy was used as standard and for labelling. The labelling procedure was the chloramine T method The average sensitivity of the assay was 4 4 pmol/l (20 pg/ml) when measuring ACTH in plasma samples The within assay coefficient of variation was 12% The between assay coefficient of variation was 21% for samples within the normal range ($n=19$)

Plasma from one patient with squamous cell carcinoma, one with oat-cell carcinoma, one with a pulmonary infiltration due to recent pneumonia, and from three control subjects was chromatographed on Sephadex G 50 medium Plasma, 3–4 ml was applied to the columns (1 6× 90 cm) and eluted with 0 1 M NaCl containing 0 25% sodium azide 0 5% 2 mercaptoethanol and 0 25% human serum albumin at 4 C Aliquots of 3 ml were collected and analysed for ACTH content Cortisol in plasma was measured by a fluorimetric technique as described by de Moor et al (14) and modified by Laurell (personal communication)

## RESULTS

Individual plasma ACTH levels are shown in Fig 1 The mean levels of total ACTH at 3 p m in plasma from patients in the cancer and lung lesion groups and from controls were 32 4±2 8 24 5±3 5 and 17 9±2 5 pmol/l respectively The mean ACTH level in the cancer group was higher than in the two other groups combined ($p<0\ 005$)

After administration of 2 mg of dexamethasone the mean decrease in plasma ACTH was significant in all groups ($p<0\ 02$ $p<0\ 001$ and $p<0\ 01$, respectively) The mean ACTH levels were 21 6±2 3, 15 5±2 0 and 13 9±2 6 pmol/l in the cancer lung lesion and control groups respectively The mean value was *significantly higher in the cancer group* than in the two other groups ($p<0\ 01$) However only six out of the 30 cancer patients had ACTH values above the range of the other two groups No difference was seen between different types of cancer (Fig 2)

Individual plasma levels of cortisol are shown in Fig 3 A slight positive correlation was found between the individual ACTH and cortisol values ($r=0\ 30$ $p<0\ 05$) The mean cortisol values before dexamethasone were 567±51 503±57 and 262±32

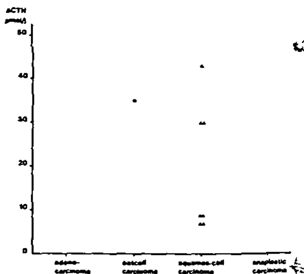



*Fig 2* Plasma ACTH levels 5 hours after administration of 2 mg of dexamethasone in patients with bronchogenic carcinoma of various types

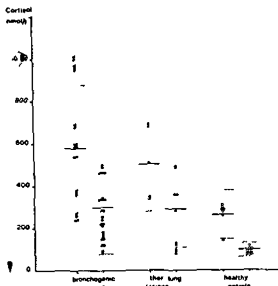



*Fig 3* Plasma cortisol levels at 3 p m in patients with bronchogenic carcinoma patients with various lung lesions and healthy controls The right column in each group shows the cortisol levels 5 hours after 2 mg of dexamethasone Symbols as in Fig 1

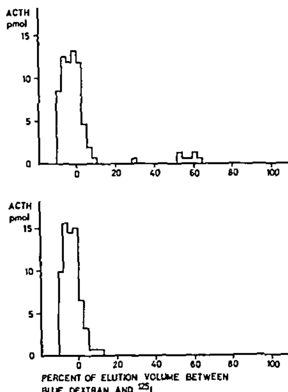



*Fig 4* Elution patterns on Sephadex G 50 medium of plasma collected at 3 p m from a patient with squamous cell carcinoma of the bronchus before (*top*) and 5 hours after (*bottom*) administration of 2 mg of dexamethasone

nmol/l in the cancer lung lesion and control group respectively the corresponding values after dexamethasone being 330±40 291±48 and 98±9 nmol/l A significant difference was found only between the controls on the one hand and bronchial cancer patients and patients with other lung lesions on the other both before and after dexamethasone The cortisol levels were comparable in the different types of cancer

At gel filtration two peaks of immunoreactive ACTH were eluted one in the void volume corresponding to big ACTH and one corresponding to labelled 1–39 ACTH The high molecular weight form was found in all subjects In a patient with pneumonia and three healthy controls the amounts of big ACTH ranged between 9 and 13 pmol/l plasma Dexamethasone administration did not change the amount of ACTH in the void volume (Fig 4) The amount of big ACTH after dexamethasone in plasma was 23 pmol/l in the patient with squamous cell carcinoma and 16 pmol/l in the patient with oat-cell carcinoma

## DISCUSSION

The mean ACTH level was significantly higher in the cancer group than in the lung lesion or control groups However several of the cancer patients had ACTH levels within the same range as the control group Our sampling technique using afternoon samples after dexamethasone administration in order to minimize the secretion of pituitary ACTH and obtain an estimate of big ACTH did not improve the discrimination between patients with bronchial cancer and controls No specific pattern due to different tumour types was revealed The results of this investigation show in accordance with those of other investigations (27 28) that determination of total immunoreactive ACTH in plasma is of limited value as a tumour marker in detecting bronchial cancer It is still possible however that repeated ACTH determinations during the course of the disease in cancer patients with elevated levels may be of value in monitoring the therapy The finding of normal ACTH levels in some patients with bronchial cancer implies that some tumours lack capacity to secrete ACTH or that the tumour must reach a certain size to be

to elaborate big ACTH in concentrations high enough to be recognized in the blood The potential capacity of non tumourous lung tissue to elaborate big ACTH is another limitation when considering the peptide as a tumour marker

Tumours derived from the bronchial mucosa have been shown to contain several amines and peptide hormones (11 20) especially ACTH and ACTH like substances (21) Before the mechanism of ACTH synthesis in lung tissue can be studied the cells able to secrete hormones and hormone homologs have to be defined Kultschitzky (9) described in 1897 granulated cells of the intestinal mucosa and postulated that they had secretory activity Like other tissues derived from the primitive alimentary tract the respiratory lining of the human lung contains cells with secretory activity (2 13 15) The histology cytochemistry and other characteristics of this cell population in the lung are well described (2 6 7 10 24 25) These cells have been included in the APUD system by the originator of the concept (16 17) It has been put forward that both the oat cell carcinoma and the carcinoid are developed from APUD cells (8 22 23)

It is more difficult to understand the mechanisms leading to production of big ACTH from bronchial tumours other than carcinoids and oat-cell carcinomas Big ACTH is however commonly found in squamous cell cancer and adenocarcinoma which are tumours derived from other cell types than those included in the APUD system

The APUD cells within dysplastic bronchial mucosa are described as exhibiting ultrastructural changes indicating altered secretory activity (5) These findings suggest the existence of a communicating system between the surface epithelium and the APUD cells within the bronchial mucosa It is not known whether this system consists of neuronal humoral or intercellular connections If the APUD cells are affected by lesion to the bronchial surface epithelium it provides one possible explanation of ACTH production in bronchial cancer as well as in other lung lesions Cell hybridization between normal neuroectodermal APUD cells and malignant non ectodermal cells has also been suggested as an explanation of the phenomenon of *ectopic hormone production* (26) More knowledge is required concerning the physiological and pathophysiological role of APUD cells in the bronchial mucosa

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## REFERENCES

1 Ayvazian L F Schneider B Gewirtz G [&] Yalow R A Ectopic production of big ACTH [in] carcinoma of the lung Am Rev Respir Dis 111 27[9] 1975
2 Bensch K G Gordon G B & Miller L R Studi[es] on the bronchial counterpart of the Kultschitzky (a[r]gentaffin) cell and innervation of bronchial glands [J] Ultrastruct Res 12 668 1965
3 Bonikos D S & Bensch K G Endocrine cells [of] bronchial and bronchiolar epithelium Am J Me[d] 63 765 1977
4 Gewirtz G & Yalow R S Ectopic ACTH produ[c]tion in carcinoma of the lung J Clin Invest 53 102[2] 1974
5 Gould V E Yannopoulos A D Sommers S C [&] Terzakis J A Neuroendocrine cells in dysplasti[c] bronchi Am J Pathol 90 49 1978
6 Hage E Endocrine cells in the bronchial mucosa [of] human foetuses Acta Pathol Microbiol Scand (A) 80 225 1972
7 — Amine handling properties of APUD-cells in th[e] bronchial epithelium of human foetuses and in th[e] epithelium of the main bronchi of human adults Act[a] Pathol Microbiol Scand (A) 81 64 1973
8 Hattori S Matsuda M Tateishi R Nishihara H & Horai R Oat-cell carcinoma of the lung Clinic[al] and morphological studies in relation to its histo[] genesis Cancer 30 1014 1972
9 Kultschitzky N Zur Frage uber den Bau des Darm kanals Arch Mikrosk Anat 49 7 1897
10 Lauweryns J M Peuskens J C & Cokelaere M Argyrophil fluorescent and granulated (peptide an[d] amine producing?) AFG cells in human infan[t] bronchial epithelium Light and electron microscop[ic] studies Life Sci (I) 9 1417 1970
11 Levine R J & Metz S A A classification [of] ectopic hormone producing tumors Ann NY Aca[d] Sci 230 533 1974
12 Liddle G W Nicholson W E & Islind D P Clinical and laboratory studies of ectopic humora[l] syndromes Recent Prog Horm Res 25 283 1969
13 McDougall J Endocrine like cells in the termina[l] bronchioles and saccules of human fetal lung a[n] ultrastructural study Thorax 33 43 1978
14 de Moor P Osinski P Deckx R & Steeno O The specificity of fluorometric corticoid determina tions Clin Chim Acta 7 475 1962
15 Owman C Håkanson R & Sundler F Occurrenc[e] and function of amines in endocrine cells producin[g] polypeptide hormones Fed Proc 32 1785 1973
16 Pearse A G E The *cytochemistry* and ultra structure of polypeptide hormone producing cells o[f] the APUD series and the embryologic physiology an[d] pathologic implications of the concept J Histochem Cytochem 17 303 1969

17 Pearse A G E & Polak J M Endocrine tumours of neural crest origin Neurolophomas apudomas and the APUD concept Med Biol 52 3 1974

18 Rassam J W & Anderson G Incidence of para malignant disorders in bronchogenic carcinoma Thorax 30 86 1975

19 Ratcliffe J J Knight R A Besser G M Lan don J & Stansfeld A G Tumour and plasma ACTH concentrations in patients with and without the ectopic ACTH syndrome Clin Endocrinol 1 27 1972

20 Rees L H The biosynthesis of hormones by non endocrine tumours—A review J Endocrinol 67 143 1975

21 — Ectopic hormone production by cancer cells Lab Lore 8 489 1978

22 Skrabanek P & Powell D Unifying concept of non pituitary ACTH secreting tumors Evidence of common origin of neural-crest tumors carcinoids and oat-cell carcinomas Cancer 42 1263 1978

23 Smith L H Oat cell carcinoma as a malignant apudoma J Thorac Cardiovasc Surg 70 147 1975

24 Tateishi R Distribution of argyrophil cells in adult human lungs Arch Pathol 96 198 1973

25 Terzakis J A Sommers S C & Andersson B Neurosecretory appearing cells of human segmental bronchi Lab Invest 26 127 1972

26 Warner T F C S Cell hybridisation in the genesis of ectopic hormone secreting tumours Lancet 1 1259 1974

27 Wolfsen A R & Odell W D ProACTH Use for early detection of lung cancer Am J Med 66 765 1979

28 Yalow R S Big ACTH and bronchogenic carcino ma Ann Rev Med 30 241 1979

29 Yalow R S & Berson S A Characteristics of big ACTH in human plasma and pituitary extracts J Clin Endocrinol Metab 36 415 1973

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# Hyperreninemia, Lysozymuria, and Erythrocytosis in Fanconi Syndrome with Medullary Cystic Kidney

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ABSTRACT Adult onset Fanconi syndrome with medullary cystic kidney was diagnosed in a 30-year old male with muscular weakness, hypokalemia, normal BP, hyperreninemia and secondary aldosteronism He also had non specific aminoaciduria, lysozymuria, and $\beta_2$ microglobulinuria Urinary concentrating and acidifying capacity was impaired and both sodium and potassium were lost into the urine I v pyelography revealed medullary cystic kidney Renal biopsy showed juxtaglomerular hyperplasia, heavy subintimal deposits and $C_3$ and IgG in preglomerular arteriolar walls, and degenerative changes in the tubules, including loss of brush border and macula densa like' lesions Polycythemia with elevated serum erythropoietin levels, and raised blood ACTH values with features of cortisolism were also present Indomethacin therapy decreased plasma renin activity (PRA), plasma aldosterone and urinary loss of potassium and sodium while serum potassium approached normal levels Metoprolol, a $\beta$ adrenergic blocking agent caused similar effects Insensitivity to the pressor effect of angiotensin II was reversed by indomethacin treatment Somatostatin infusion lowered PRA and aldosterone without affecting BP Several biochemical aberrations of this patient resemble Bartter s syndrome including the effect of indomethacin

*Key words* Fanconi syndrome renin aldosterone polycythemia somatostatin

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Fanconi syndrome of the idiopathic adult onset type (30) is a tubulopathy of unknown cause characterized by excessive urinary loss of amino acids monosaccharides electrolytes proteins and water (1 30) Increased urinary loss of lysozyme insulin and vitamin D may also occur (1 13 26) The loss of solutes is due to reduced net tubular reabsorption but tubular secretion is also impaired An autosomal recessive pattern of inheritance has been proposed (30) Secondary Fanconi syndrome has been described following heavy metal poisoning in Wilson s disease plasmocytoma galactosemia tyrosinosis tetracycline poisoning and in relation to poisoning with aromatic compounds (1 30)

Medullary cystic kidney thought to belong to the same disease entity as juvenile nephronophtisis (19 23) and to be different from others (4) is characterized by multiple small cysts up to 10 mm in diameter in the outer medulla less frequently in the cortex (35) Renal tubular destruction is thought to be primary while interstitial nephritis is considered secondary The cysts are believed to arise from collecting ducts (16 35) Hyperplasia of the juxtaglomerular apparatus (JGA) has been reported in 5 out of 9 patients with juvenile nephronophthisis (4)

Bartter s syndrome consists of hyperreninemia with secondary aldosteronism normal blood pressure and insensitivity to the pressor effect of angiotensin II (2 22) A tubular defect is believed to cause Bartter s syndrome (2 10)

We describe a patient with Fanconi syndrome of the idiopathic adult onset type who also has medullary cystic kidneys and several features of Bartter s syndrome

## CASE REPORT

A previously healthy 30-year-old male gipsy height 170 cm weight 78–84 kg was repeatedly examined since 1967 because of periodic muscular weakness potassium losing nephropathy and polycythemia He had polyuria averaging 3 500 ml daily Serum potassium concentration was 1 6–2 8 mmol/l without medication and 2.5–4 1 mmol/l with potassium supplement

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*Abbreviations* PRA=plasma renin activity BP=blood pressure JGA=juxtaglomerular apparatus ACTH=adrenal corticotropic hormone

Table I *Effects of indomethacin and metoprolol (mean values)*

| Drug[a] | Serum Na+ (mmol/l) | Serum K+ (mmol/l) | Serum lysozyme (µg/ml) | Urinary lysozyme (µg/ml) | PRA (ng ml⁻¹ h⁻¹) Supine | Upright |
|---|---|---|---|---|---|---|
| None | 143 | 2 6 | 19 0 | 7 4 | 15 4 | 41 8 |
| Indomethacin 75 mg/d | 142 | 3 9 | 12 6 | 2 7 | 6 9 | 10 2 |
| Metoprolol 100 mg/d | 143 | 3 4 | 20 2 | 18 5 | 6 2 | 9 4 |
| Reference values in healthy subjects on free diet | | | 3–9 | <2 0 | 0 9–2 0 | 2 0–5 0 |

[a] Continuous potassium supplement was 230 mmol/d

In 1973 i v pyelography suggested medullary cystic kidney There was non specific aminoaciduria without cystinuria and intermittent glycosuria In 1976 central obesity and abdominal striae appeared along with palmar hyperpigmentation Serum cortisol levels and urinary excretion of 17-hydroxycorticoids and 17 ketosteroids remained normal Two bone marrow aspirates showed signs of secondary polycythemia

On admission in Jan 1977 moderate features of cortisolism were noticed There was slight muscular weakness Gross neurologic findings were normal Blood pressure (BP) averaged 120/80 mmHg The patient admitted regular smoking and moderate to heavy intake of alcoholic beverages and denied exposure to heavy metals cadmium or lysol

v pyelography showed numerous 1–20 mm wide enlargements of the collecting ducts X rays of the skull sella and chest were normal Nephrography with $^{131}$I hippuran was normal Renal creatinine clearance was 1 41 ml sec⁻¹ m⁻²

Throughout the study the diet contained 230 mmol of potassium chloride daily as a supplement and 100–160 mmol of sodium chloride

*Serum and urinary electrolytes*

Potassium balance was negative Whole body counting revealed low total body potassium 1 43 g/kg (normal mean±S D for age and sex 2 06±0 24) During indomethacin treatment (Table I) serum K+ values normalized along with a sharp drop in urinary excretion of K and Na During treatment with metoprolol 50 mg twice times daily serum K+ rose slightly (Table I) The following serum concentrations were normal $Ca^{++}$ 2 5–2 3 mmol/l inorganic P 0 9 mmol/l Cl 99–105 mmol/l creatinine 85–98 µmol/l Blood pH was 7 39–7 42 pCO 32–39 mmHg $HCO_3$ 19 5 mmol/l Serum osmolality was moderately raised 306–314 mmol/l and blood glucose was raised 8 7–11 6 mmol/l

*Renal functional variables*

Urine was alkaline pH 7 0–8 2 and remained so during ammonium chloride load Urinary concentrating ability was impaired maximal urinary osmolality achieved after 20 hours of water deprivation being only 373 mOsmol/kg

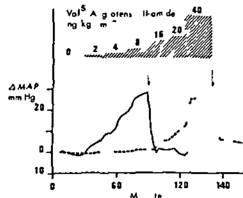



*Fig 1* Change in mean arterial pressure (*MAP*) in response to increasing doses of val⁵ angiotensin II amide infusion (supine position) before ( ) and after (—) treatment with indomethacin Arrows indicate discontinuation of infusions

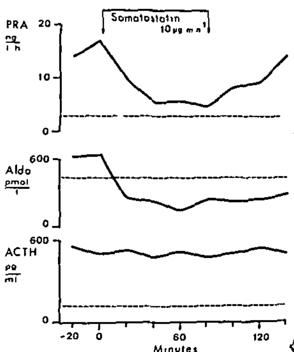



*Fig 2* Effect of somatostatin infusion in the supine position on PRA plasma aldosterone and ACTH concentration = Upper limits of reference values

| Plasma aldosterone (pmol/l) | | Serum angiotens n I convert ng enzyme (µmol mun l ) |
|---|---|---|
| Sup ne | Upright | |
| 1 312 | ? 370 | 61 5 |
| 318/ 643 | | 42 4 |
| 305/ 655 | | 56 2 |
| 70–55 | 95–530 | ?? 1 34 3 |

H O Urinary specific grav ty ranged from 1 009 to 1 012 Phenol red excret on was normal 40% excreted n one hour There was a pers stent lysozymuria (Table I) and serum lysozyme concentrat ons (w th lysoplate techn que) were raised (Table I) Treatment w th indomethac n 25 mg 3 times daily lowered both urinary and serum lysozyme levels Urinary concentrat on of $\beta_2$-microglobu l n (Pharmac a $\beta_2$ m croglobul n kit) was greatly n creased 34 0 µg/l (normal below 0 37) Prote nuria of 0 19–0 70 g/l was present SDS d sc electropho es s of urine concentrated ?00 t mes against polyv nyl pyrrol done revealed major bands w th molecular we ghts of about 70 000 daltons and lower cons stent w th tubular prote nuria Non spec f c am noac duria w thout cys t nuria was found tw ce Treatment w th the long act ng vasopress n analogue desam no-D-arg n ne vasopressin (M n rin Ferring) 0 µg ntranasally d d not affect uri nary output and urinary osmolal ty rose only from 340 to 375 mOsmol/kg H O

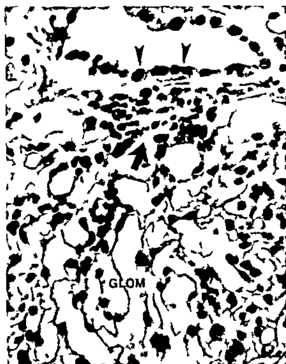
*Ren n–aldoste one ax s*

The renin–aldosterone system was markedly stimulated (Table I) Both indomethac n and metoprolol therapy (Table I) reduced plasma renin act v ty (PRA) (7) and plasma aldosterone (Cea Ire Sorin kit) as shown n Table I Serum angiotens n-converting enzyme ( 0) was raised (Table I)

Res stance to the pressor act on of val$_5$ angiotens n II amide (Hypertens n C ba) d ssolved in 5% glucose was d min shed by ndomethac n treatment (F g 1) 75 mg daily for 5 days Somatostatin (Ferring International Malmö Sweden) was infused i v at a rate of 10 µg min (800 µg/100 ml 5% glucose) for 80 min PRA and plasma aldosterone were marked y lowered during somatostat n nfus on (F g 2) Plasma adrenal corticotrop c hormone (ACTH) (Amersham ACTH kit England) remained high during somatostatin nfus on

*O l er hormone stud es*

Serum cortisol levels were normal 0 34–0 41 µmol/l Uri nary free cortisol excretion was 0 01–0 19 µmol/?4 h (normal 0 ?8) Serum concentra on of testosterone 12 4 nmol/l (normal 14–33) was sl ghtly subnormal Serum parathyro d hormone concentrat on determined by ra d o mmunoassay was normal 0 33 ng/ml (normal 0 54) and urinary 24 hour exc etion of cycl c AMP ? 3 µmol/l (normal 2 1 5 6) were normal Serum concentrations of




F g 3 Light m crograph of the h lar region of a glomerulus (*GLOM*) show ng hyperplas a of the JGA (arrow) Arrowheads nd cate macula densa of the distal tubule H&E ×470

rad oimmunoassayable vasopressin (8) were nappropriately h gh n the hydrated state 7 6–8 3 pg/ml (normal 2 0+1 2) and rose to 11 8 pg/ml after 0 hours of water depriv ation and were ncompletely suppressed following water load 3 3–4 1 pg/ml

*Hema olog cal f ndings*

Hemoglob n was 181 07 g/l packed red cell volume 41 2 ml/kg b wt (normal 28–35) Arterial $pO_2$ was normal Serum erythropo et n levels measured w th a hemagglut nat on nh b tion tech que (JCL Knoxv lle Tennessee) were ra sed 2 0– 80 m lli mmunochem cal un ts (normal below 75) Blood leukocyte counts 9 6–16 7 10$^9$ 1 and platelet counts 58–534 10$^9$ 1 we e ele ated Bone marrow asp ra e showed s gns of secondary polycy them a. Karyotype was normal 46 XY w thout chromosomal aberrations

*Renal morpholog*

Renal b opsy showed hyperplasia of the JGA (F g 3) and nterstitial f bros s Electron microscopy revealed exten s ve sub numal depos ts n renal preglomerular arterioles (F g 4) and degenerati e changes of proximal and d stal tubular ep helium w th lamel ary th ken ng of the base ment membrane and part al loss of brush border (F g 5) The e were numerous acuoles n the tubular ep thel al cells A les on resembling the juxtaglomerular macula




*Fig 4* Electron micrograph of a part of a glomerulus (bottom) and its arteriole (*A*) The arteriolar wall is extensively thickened due to deposition of electron-dense fine granular material (*DEP*) (hyaline arteriosclerosis) The glomerular capillary wall (*CAP*) is normal *BC*=Bowman s capsule ×370

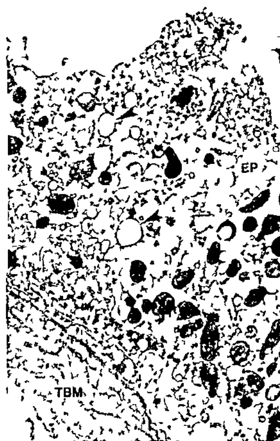



*Fig 5* Electron micrograph of a part of a tubule sho profound vacuolization (arrowheads) of the tub epithelium (*EP*) and lamellar thickening of the tub basement membrane (*TBM*) ×7360

densa with numerous cells containing scanty cytoplasm and hyperchromatic nuclei was observed in the distal tubular wall (Fig 6)

## DISCUSSION

The similarities of our patient s clinical picture with Bartter s syndrome namely hyperreninemia with hyperplastic JGA aldosteronism normal BP and insensitivity to the pressor effect of angiotensin II which could be reversed by indomethacin treatment (3 34) are striking These features therefore appear not to be confined to Bartter s syndrome They may be associated with a variety of tubulopathies associated with loss of electrolytes

Renal tubulopathy was established by aminoaciduria of the non specific pattern corresponding to idiopathic adult onset Fanconi s drome (1 30) Lysozymuria reported to occu Fanconi syndrome (13 26), was diminished for known reasons during treatment with indome cin In our patient the lysozyme clearance 2 1–2 8 ml/min with a lysozyme/creatinine cl ance ratio of 3 0–5 1% values that are in g agreement with those reported by Pruzanski Wilson (26) in three patients with adult onset F coni syndrome

In addition to lysozymuria the 90 fold ris urinary excretion of $\beta_2$ microglobulin indicated fective proximal tubular reabsorption of molecular weight proteins Excretion of these molecular weight proteins (MW 14000–14500 tons) occurs in proximal tubular dysfunction sociated with cadmium poisoning which also cause of secondary Fanconi syndrome Howe urinary excretion of cadmium lead α ami




*F g 6* Light micrograph of a tangen ally sec oned glomerulus (*GLOM*) showing the proximal part of the proximal tubule l ned by abnormal macula densa like ep thel um (arrowheads) H&E ×4 0

levul n c ac d and cobalt were normal n our pa t ent

Leucocytosis n the absence of renal insuff cien cy is the main contr butor to elevated serum lysozyme concentrat on (18) Lysozyme is normally totally reabsorbed from the glomerular ultrafiltrate very low amounts being present n the urine Lysozymuria occurs when the f ltered loss of lysozyme exceeds the max mum transport cap c ty of the prox mal tubules or from a decreased ab l ty of the prox mal tubules to remove lysozyme from the glomerular filtrate or from a d rect les on of the proximal tubular cells Lysozymuria has been con s dered an ev dence of abnormal tubular funct on f the serum lysozyme is below 45 μg/ml or 3–5 times above the normal serum lysozyme concentrat on (14 15 ?6) as n our pat ent

Lysozymuria together w th increased urinary potass um and low serum potass um levels has re peatedly been demonstrated n monocytic and myelomonocytic leukem a pat ents w th h gh serum lysozyme levels (14 ?4 ?5) The mechan sm beh nd th s relat onsh p s not fully understood Both lysozyme and hypokalem a per se have been thought to cause tubular les ons (13 3?) In fact perfusion studies with isolated rat kidney have shown that lysozyme increases potass um excretion (?1)

In Fanconi syndrome there is urinary loss of sodium and potass um due to tubular dysfunct on as observed in our pat ent Tubulopathy was also re flected by inab l ty to ac d fy and concentrate urine Resistance to the long act ng vasopress n analogue desam no-D-argin ne vasopress n together with moderate polyuria lo v urinary osn olal ty and hyposthenuria desp te endogenous plasma vaso-pressin levels suff cient to produce ant d ures s n normal man po nts to disturbed funct on of the renal medullary targets of vasopress n Th s may be due to mpa red osmotic grad ent of the medulla and to degenerative changes in distal tubules and collect ng ducts assoc ated w th medullary cyst c k dney

In Bartter s syndrome tubular cell vacuol zation medullary cysts and loss of brush border are l ck ing (6) and lysozymuria is absent (6) Th s sets Bartter s syndrome apart from the aberrat ons found in our pat ent

Heavy sub nt mal depos ts in the renal arter oles (F g 5) and the presence of $C_3$ and IgG in the structures may nd cate that arteriolar w ll le t is assoc ated with depos tion of in n ine c plexes play a role n Fancon syndron e V e l t v ef fects of long last ng hyperreninemia (9) poss l ly re lated to th s observ ti r p t ent ren a nterest ng poss b l ty Hyperplas a of the JGA in our pat ent ccords with recent observat ons n 5 out of 9 pat ents w th juven le nephronophth s s (4) a cond t on poss bly belong ng to the same e tity as medullary cyst c k dney (19 23) The m c l densa l ke les on (F g 4) observed in the d st l t u lar wall was reported by Sherman et al (31) t e larger and more abundant n juven le n th s s than n other atroph c renal dise se ch nge may represent compensatory h perplasia of the tubules (31)

The markedly st mulated ren n n our pat ent was probably due t lytes Res stance to the press It was s m lar to that ol serv drome (2 6 34) and n a variety cond tions Treatment w PRA and plasma aldoster s um balance reverted concentrat ons normal

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# No Effect of Cimetidine on Calcitonin Secretion from Medullary Thyroid Carcinoma

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**ABSTRACT** The effect of cimetidine on basal and pentagastrin stimulated serum immunoreactive calcitonin (S-iCT) concentrations was studied in six patients with medullary carcinoma of the thyroid (MCT) Basal S-iCT was elevated in all patients and showed a marked increase after i v injection of pentagastrin, 0 5 µg/kg b wt Cimetidine, 200 mg i v 30 min before administration of pentagastrin had no effect on either basal or pentagastrin-stimulated S-iCT Thus, the mechanisms of basal and pentagastrin stimulated calcitonin secretion from MCT do not seem to involve agonism with histamine $H_2$-receptors

*Key words* serum calcitonin medullary cancer of the thyroid pentagastrin stimulation cimetidine

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The secretion of calcitonin from medullary thyroid carcinoma (MCT) is stimulated by pentagastrin (8) Nothing is however known about its mode of action Pentagastrin also stimulates gastric acid secretion Here its mode of action involves a direct binding to gastrin receptor sites on the parietal cell and/or a local increase in the availability of histamine (3) In either case some agonism with histamine on the parietal cell is necessary as blockade of histamine $H_2$ receptor sites with cimetidine strongly inhibits gastric acid secretion after pentagastrin (3) MCT contains the enzymes L aromatic amino acid decarboxylase (2) and histaminase (1) and thus has the biochemical potential for producing and degrading histamine In analogy with the concepts of gastric acid secretion the mechanisms of calcitonin secretion from MCT might implicate some agonism with histamine $H_2$-receptor sites on the neoplastic cells

In order to test this hypothesis we investigated the effect of cimetidine on basal and pentagastrin stimulated serum immunoreactive calcitonin (S iCT) concentrations in six patients with MCT

## PATIENTS AND METHODS

Three female patients aged 34–72 years and three male patients aged 30–50 years with MCT were studied Informed consent to participate was obtained from all patients All had elevated basal S iCT concentrations (Table I) In two patients with familial occurrence of MCT the diagnosis was verified histologically by a subsequent thyroidectomy Four patients with sporadic disease were previously operated on for MCT Persistently elevated basal postoperative S-iCT indicated metastatic disease

S-iCT was measured by a radioimmunoassay (6 7) using a commercial antibody to synthetic human calcitonin (Calbiochem USA) and synthetic human monomer calcitonin (supplied by Ciba Switzerland) for standards and iodination Assay conditions were modified according to the method of Dietrich et al (5) The detection limit was 20 pg/ml normal range 0–120 pg/ml detectable [illegible] values could be found in 65% of normal subjects [illegible] intra assay coefficient of variation was below [illegible] *serum concentration of 350 pg/ml* and 14 [illegible] concentration of 30 pg/ml The interassay variation [illegible] ficient was 14% at a S-iCT concentration of [illegible]

All investigations were started at 9 a m [illegible] *night fast Blood samples were drawn via* [illegible] la. In the first experiment S-iCT was [illegible] subjects before and 2 5 15 and 30 [illegible] injection of pentagastrin (Peptavlon®) [illegible] diluted in 1–2 ml isotone sodium [illegible] sec The second experiment was [illegible] later S-iCT was measured before [illegible] after (2 5 15 27 and 29 min) an i v [illegible] (Tagamet®) 200 mg given with [illegible] injected i v 30 min after cimetidine [illegible]

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*Abbreviations* MCT=me[illegible] roid S-iCT=serum imm[illegible]

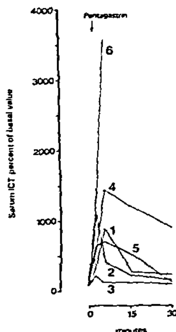



*Fig 1* S-iCT levels before and after i v injection of pentagastrin 0.5 µg/kg b wt (experiment I) The 15- and 30-min values of patient 6 are lacking.

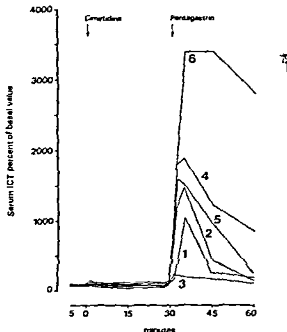



*Fig 2* S-iCT levels before and after i v injection [of] cimetidine 200 µg and pentagastrin 0 5 µg/kg b wt ([ex]periment II)

ment. S-iCT was subsequently measured 32 35 45 and 60 min after the cimetidine injection

The statistical significance of differences in group means was determined by Wilcoxon s test for two samples or for paired samples

## RESULTS

Table I gives the basal S-iCT levels in the 6 patients with MCT before the first and second experiment *No significant difference was found between the* values

The effect of i v pentagastrin on S-iCT levels is

Table I *Basal S iCT levels at the time of the first (I) and second (II) experiment (normal range 0–120 pg/ml)*

| Pat. no | S-iCT (pg/ml) I | II |
|---|---|---|
| 1 | 110 000 | 93 000 |
| 2 | 3 000 | 2 300 |
| 3 | 1 600 | 1 400 |
| 4 | 2 200 | 1 875 |
| 5 | 350 | 300 |
| 6 | 9 000 | 9 500 |

shown in Fig 1 All patients demonstrated a ri[se] ($p<0\ 05$) in S-iCT the maximal stimulated valu[es] reaching 230–3 600 % of basal S-iCT levels

Fig 2 shows the effect of cimetidine on basal a[nd] pentagastrin stimulated S iCT levels Cimetidi[ne] had no significant effect on basal S iCT concentr[a]tions and the rise in S-iCT ($p<0\ 05$) followi[ng] pentagastrin 30 min later was of the same order [of] magnitude as following pentagastrin alone in t[he] first experiment.

## DISCUSSION

C-cells have a biochemical potential to produce an[d] degrade histamine (1 2) and other amines, i[.e.] adrenergic agents and dopamine may stimulate o[r] inhibit calcitonin secretion (2 4)

The present investigation demonstrated th[at] cimetidine had no acute effect on basal and pent[a]gastrin stimulated S-iCT levels in patients wi[th] MCT The dose of cimetidine administered in th[e] present study would produce a significant histamin[e] $H_2$-receptor blockade on the gastric parietal cel[ls] for at least two hours (9) This interval exceeds th[e] 30 minutes between pentagastrin and cimetidine i[n] this study The mechanisms behind calcitonin sec

retion seem therefore not to involve binding of histamine to $H_2$ receptors on C cells

Due to the limited sensitivity of most calcitonin radioimmunoassays information about factors regulating calcitonin secretion has largely been obtained by studying MCT patients as in the present study Most results of these investigations have however been confirmed in normal human subjects using very sensitive calcitonin assays (4)

## ACKNOWLEDGEMENT

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## REFERENCES

1 Baylin S B Beaven M A Engelman K & Sjoerdsma A Elevated histaminase activity in medullary carcinoma of the thyroid gland N Engl J Med 283 1239 1970
2 Baylin S B Hsu T H Stevens S A Clayton H Kallman C H Trump D L & Beaven M A The effects of L-dopa on in vitro and in vivo calcitonin release from medullary thyroid carcinoma Clin Endocrinol Metabol 48 408 1979
3 Burland W L & Mills J G Histamine $H_2$-receptor blockade and treatment of duodenal ulcer In Gut hormones (ed S R Bloom) p 638 Churchill Livingstone Edinburgh London and New York 1978
4 Deftos L J Roos B A Knecht G L Lee J C Pavlinac D Bone H G & Parthemore J G Calcitonin secretion In Endocrinology of calcium metabolism (ed D H Copp & R V Talmage) p 134 Excerpta Medica Amsterdam and Oxford 1978
5 Dietrich F M Hunziker W H & Fischer J A Synthetic human calcitonin Analysis of antibodies obtained from various animal species and determination of immunoreactive hormone in human sera Acta Endocrinol (Kbh) 80 465 1975
6 Nielsen H E Christensen C K & Olsen K J Serum calcitonin in patients with chronic renal disease Acta Med Scand 205 615 1979
7 Nielsen H E & Olsen K J Serum calcitonin after renal transplantation Acta Med Scand 205 619 1979
8 Telenius-Berg M Almquist S Berg B Hedner P Ingemanssen S Tibblin S & Wasthed B Screening for medullary carcinoma of the thyroid in families with Sipple s syndrome evaluation of new stimulation tests Eur J Clin Invest 7 7 1977
9 Walkenstein S S Dubb J W Randolph W C Westlake W J Stote R M & Intoccia A P Bioavailability of cimetidine in man Gastroenterology 74 360 1978

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# Sucrose and Sorbitol as Sweeteners in the Diet of Insulin-Dependent Diabetics

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**ABSTRACT** Blood glucose levels following breakfast meals containing sorbitol or sucrose as sweeteners were investigated Nine insulin treated diabetics received two test meals after an overnight fast The meals were composed of 90 g white bread, 9 g butter and 100 g stawberry jam which on one occasion contained 18 g sorbitol as sweetener and on another 18 g sucrose Blood glucose was measured for three hours following the meal The test meal sweetened with sucrose showed a slightly faster postprandial rise in blood glucose than the sorbitol sweetened meal but this difference is not statistically significant at any point of the curves Taking into consideration that sorbitol has a sweetening effect of only 60% of that of sucrose, we conclude that neither sucrose nor sorbitol are acceptable sweeteners for insulin dependent diabetics

*Key words* insulin-dependent diabetes diet sweeteners sorbitol sucrose

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We do not allow sucrose in the diabetic diet Firstly it is regarded as a rapidly absorbable carbohydrate and secondly it is not an essential nutrient exclusion of its leads to a decrease in the energy intake However since most diabetics want some sweetened foods in their diet different kinds of sucrose substitutes have been used

The sugar alcohol sorbitol has been used since 1929 as a sweetener for the diabetic patient (12) It has been stated that among its properties are the following it is a tasty sweetener it is absorbed slowly—therefore metabolized slowly—and gives no peak loading its metabolism is to a large extent independent of insulin and it has no significant effect on the blood glucose level (2 3 4 8 10 11) However few studies have been carried out in insulin-dependent diabetics Arvidsson Lenner (1) gave nine adult-onset diabetics a base diet supplemented with applesauce sweetened with sucrose fructose or sorbitol (Sorbitol comprised 32% of the total carbohydrate content of the meals ) No significant differences were found between sorbitol fructose and sucrose-containing meals with respect to the effect on the blood glucose level or on glucosuria

We therefore studied the blood glucose responses after test meals sweetened with sucrose or sorbitol in young insulin-dependent diabetics

## PATIENTS AND METHODS

The study is based on experiments with nine insulin treated juvenile diabetics Table I shows relevant patient data All patients received insulin twice daily

Each person was given two different breakfast test meals in which jam on one occasion was sweetened with 18 g sucrose (+ 0 02 g saccharin) and on another with 18 g sorbitol (+ 0 02 g saccharin) The composition of the test meals is given in Table II 27% of the total carbohydrate content was sweetener The test meals were given after an overnight fast and were consumed in 10 min The test persons had taken their normal insulin dose in the preceding afternoon but not in the morning of the test day

Capillary blood samples were taken in the fasting state and 0 15 30 60 90 120 150 and 180 min after the meal Blood glucose was determined enzymatically with glucoseoxidase (Glox)

Wilcoxon s test for paired differences was used in the statistical calculations

## RESULTS

Fig 1 shows the average increase in blood glucose after the two different breakfast meals

The results show that the increase in blood glucose is slightly faster after the sucrose sweetened than after the sorbitol sweetened meal but this difference is not statistically significant at any point of

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Table I *Relevant patient data*

| | Mean | Range |
|---|---|---|
| Age (y ) | 21 | 16–30 |
| Duration of diabetes (y ) | 6 5 | 3–11 |
| *Insulin requirement (IU)* | | |
| Morning | | |
| NPH | 29 | 16–48 |
| Rapid acting | 13 | 2–44 |
| Afternoon | | |
| NPH | 24 | 12–44 |
| Rapid acting | 7 | 4–10 |

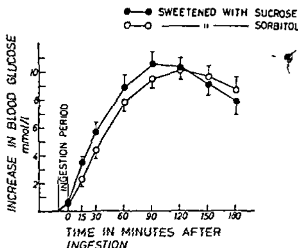



*Fig 1* Mean increase (±S E M ) in blood glucose after the two test meals

the curves The maximum peak values of glucose after the two meals did not show any statistically significant difference 10 6±0 8 mmol/l above the fasting value after the sucrose sweetened and 10 1±0 6 mmol/l after the sorbitol sweetened meal

## DISCUSSION

It is interesting that whether we used sorbitol or sucrose as a sweetener in our mixed test meals no significant difference could be seen in the postprandial blood glucose responses of the insulin-dependent diabetic test persons This is in good accordance with the observation of Arvidsson Lenner (1) who studied adult onset diabetics

The majority (more than 80%) of the absorbed sorbitol is taken up in the liver where it is metabolized via fructose Fructose can then be metabolized to pyruvate and Krebs cycle intermediates or to glucose/glycogen depending on whether glycolysis or gluconeogenesis dominates (5) Absence of insulin will stimulate gluconeogenesis while insulin in adequate amounts favours glycolysis with production of Krebs cycle intermediates (6) Our patients did not receive their ordinary morning insulin dose before the test meals but took their insulin in the evening before (mainly NPH insulin) The work by Kolendorf et al (7) shows that there is a significant blood glucose lowering effect of subcutaneously injected NPH insulin after 1 hours Our test persons might have been in a stat of relative insulin depletion However many insulin dependent diabetics are treated with only on injection of NPH insulin in the morning and the might be definitely more insulin-depleted tha our patients

Sucrose is composed of one part of glucose an one part of fructose The latter is absorbed mor slowly than the former and is to a large exter metabolized in the liver as previously discussed (6 The main product of sorbitol and fructose in ou test meals seems to be glucose which is subsequen ly released into the circulaton

The sweetener comprised 27% of the total ca bohydrate content of our test meals a figure we above that in ordinary meals of our insulin depend ent diabetics

The relative sweetening effect of sorbitol an

Table II *Composition of the test meals*

| | Weight (g) | Carbohydrate including sweetener (g) | Fat (g) | Protein (g) | Energy (kJ) |
|---|---|---|---|---|---|
| White bread | 90 | 44 | 2 2 | 7 3 | 941 |
| Butter | 9 | tr | 7 4 | tr | 281 |
| Strawberry jam | 100 | 22 | tr | 0 4 | 349 |
| Total | 199 | 66 | 9 6 | 7 7 | 1 571 |

tr = trace

sucrose have not been taken into consideration in this study Sorbitol is usually said to have a sweetening effect of only 50–60% of that of sucrose (5) Taking that into consideration our conclusion must be that neither sucrose nor sorbitol are feasible sweeteners for insulin-dependent diabetics

## REFERENCES


1 Arvidsson Lenner R Specially designed sweeteners and food for diabetics—a real need? Am J Clin Nutr 29 726 1976
2 Bundgard G Die Zuckeraustauschstoffe Teil II b Dtsch Apoth 23 429 1971
3 — Die Zuckeraustauschstoffe Teil III c Dtsch Apoth 24 171 1972
4 Forster H Comparative metabolism of xylitol sorbitol and fructose In Sugars in nutrition (ed H L Sipple & K W McNutt) p 259 Academic Press New York San Francisco and London 1974
5 Hue L The metabolism and toxic effects of fructose In Sugars in nutrition (ed H L Sipple & K W McNutt) p 357 Academic Press New York San Francisco and London 1974
6 Huttunen J K Fructose in medicine A review with particular reference to diabetes mellitus Postgrad Med J 47 654 1971
7 Kølendorf K Aaby P Westergaard S & Deckert T Absorption effectiveness and side effects of highly purified porcine NPH insulin preparation (Leo®) Eur J Clin Pharmacol 14 117 1978
8 Mehnert H Zur parenteralen und oralen Applikation der Zuckeraustauschstoffe Fructose Sorbit und Xylit bei Diabetikern Med Ernahr 11 77 1970
9 Moskowitz H R The psychology of sweetness In Sugars in nutrition (ed H L Sipple & K W McNutt) p 37 Academic Press New York San Francisco and London 1974
10 Ruttloff H & Ketz H A Zur Resorption von Sorbit und Mannit Die Nahrung 5 599 1961
11 Steinke J Wood Jr F C Domenge L Marble A & Renold A E Evaluation of sorbitol in the diet of diabetic children at camp Diabetes 10 218 1961
12 Thannhauser S J & Meyer K H Sorbit (Sionon) als Kohlenhydratersatz für den Diabeteskranken Munch Med Wochenschr 76 356 1929

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# The Effects of Long-Term Antithyroid Drug Treatment on Serum Reverse T3 in Patients with Graves' Disease

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**ABSTRACT The effects of long term treatment with antithyroid drugs, carbimazole (CMI) or propyl thiouracil (PTU), on serum reverse triiodothyronine (rT3) levels were studied in 23 patients with Graves' disease Nineteen patients were given CMI and four PTU for a minimum of six months After one month of treatment the serum levels of thyroxine (T4), triiodothyronine (T3) and rT3 had normalized in both groups When L-thyroxine was added to the regimens after two months of therapy, both serum T4 and rT3 levels increased, whereas serum T3 level continued to fall *The serum levels of rT3 seemed to* be dependent on and followed the T4 levels so closely that determinations of rT3 in the medical management of patients with Graves disease will be of little clinical use**

*Key words* antithyroid drug hyperthyroidism reverse triiodothyronine

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During antithyroid drug treatment of thyrotoxicosis the clinical evaluation of the patient may be an unreliable indicator of the thyroid hormone levels and chemical euthyroidism can exist long before clinical euthyroidism (11) Repetitive thyroid hormone determinations are therefore necessary to estimate the antithyroid drug effect Serum determinations of reverse triiodothyronine (rT3) have resulted in a better understanding of thyroid hormone physiology (3) and antithyroid drug pharmacology (8) but have so far not been employed much in diagnostic or clinical practice (17)

In this study measurements of thyroid hormone levels with special reference to rT3 were evaluated in thyrotoxic patients on long term treatment with carbimazole (CMI) and propyl thiouracil (PTU) A point of particular interest was the extent to which rT3 determinations throughout the treatment period would provide additional information for the chemical evaluation of the patients

## PATIENTS AND METHODS

*Twenty three patients* (three men and 20 women age range 23–69 years) with Graves disease were studied The diagnosis was based on clinical symptoms elevated serum levels of triiodothyronine (T3) and thyroxine (T4) absent TSH response to a TRH test increased diffuse thyroid uptake of $^{99m}TcO_4$ and finally neither findings at fine needle biopsy of the thyroid nor thyroid antibody titers suggesting thyroiditis

The antibodies were measured by routine assays antithyroglobulin titers with a tanned red cell reagent kit (Burroughs Wellcome) and antimicrosomal titers with an immunofluorescence technique Antithyroglobulin titers above 1/1280 and/or antimicrosomal titers above 1/100 strongly suggest thyroiditis

Nineteen patients were treated with CMI (Neo-Mercazole®) and four with PTU (Tiotil®) The treatments were assigned according to the initial serum levels of T3 Thus patients with T3≤4 0 nmol/l before therapy were given 5 mg×3 of CMI ($n$=2) those with T3 between 4 1 and 9 9 nmol/l were given 10 mg×3 of CMI ($n$=14) or 100 mg×3 of PTU ($n$=3) and patients with T3≥10 0 nmol/l were given 10 mg×4 of CMI ($n$=3) or 100 mg×4 of PTU ($n$ 1) After one month these regimens were changed in all cases to 5 mg × 3 of CMI or 50 mg × 3 of PTU respectively After yet another month 0 1 mg of L thyroxine (Levaxin®) was added and the patients continued on this treatment for varying periods up to two years No patient received $\beta$ blocking or glucocorticoid drugs

### *Hormone assays*

*T3 in serum* was measured by solid phase radioimmunoassay with antibodies coupled directly to microcrystalline cellulose particles activated with cyanogen bromide (20) Incubations were performed at 60°C for two hours and then overnight at room temperature in 0 5% polysorbate 20 (Tween 20) in 0 05 M phosphate buffered saline pH 7 4

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*Abbreviations* T4 = thyroxine T3 = triiodothyronine rT3 = reverse T3 FT4-I = free T4 index CMI = carbimazole PTU = propylthiouracil

16 Ratcliffe W A Marshall J & Ratcliffe J G The radioimmunoassay of 3 3 ,5 triiodothyronine (reverse T3) in unextracted human serum Clin Endocrinol 5 631 1976
17 Schimmel M & Utiger R D Thyroidal and peripheral production of thyroid hormones and their clinical implications Ann Intern Med 87 760 1977
18 Sterling K Refetoff S & Selenkow H A T3 thyrotoxicosis thyrotoxicosis due to elevated serum triiodothyronine levels JAMA 213 571 1970
19 Taurog A The mechanism of action of the tioureylene antithyroid drugs Endocrinology 98 1031 1976
20 Wide L Radioimmunoassays employing immunosorbents Acta Endocrinol (Kbh) (Suppl) 142 207 1969

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# Unique Antigenic Determinants (Idiotypes) Used as Markers in a Patient with Macroglobulinemia and Urticaria

*Similar Idiotypes Demonstrated in the Skin and on Peripheral Blood Lymphocytes*

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ABSTRACT An antiserum was raised against a monoclonal IgMκ macroglobulin isolated from serum of a patient with recurrent urticaria The antiserum was made idiotype-specific through adequate absorptions The anti idiotype antiserum reacted *only with the immunizing protein and its Fab fragments* and not with other monoclonal proteins of IgM and IgA class or pooled IgG as assayed in an enzyme linked immunosorbent assay IgM antibodies with the same idiotype as the monoclonal IgM protein were detected in the dermal/epidermal junction area of diseased skin The similar idiotypic determinants could also be demonstrated on membrane bound molecules of peripheral blood B and T lymphocytes using the immunofluorescence methods

*Key words* idiotypes macroglobulinemia urticaria

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Anti idiotype antisera are highly specific for antigenic determinants in or near the antigen binding sites of immunoglobulins These antigens are called idiotypic determinants or idiotypes (4) and these determinants can be used as markers for certain antibody populations

Shared idiotypes between serum immunoglobulins and membrane bound molecules on peripheral blood B and T lymphocytes have recently been detected in humans (5 9 11 12 15 16) In the present study an anti idiotype antiserum raised against a monoclonal IgMκ protein from a patient with macroglobulinemia and urticaria was used to examine skin biopsies and peripheral blood B and T lymphocytes from the patient

## MATERIALS AND METHODS

*The patient* A 52 year-old man hospitalized at the Department of Dermatology Rikshospitalet Oslo had a 4 years history of recurrent urticarial wheals lasting up to 2–3 days Since the onset he had never been free from lesions During the last 3 years the patient has had migrating arthralgias mainly involving the limbs During long periods his body temperature was elevated to about 38°C The patient had used no drugs before the onset of symptoms and he had had no serious primary diseases

The ERS varied from 50 to 140 mm/h The leucocyte counts were 7000–14000/mm³ Hb liver function tests and creatinine were all normal The quantitation of immunoglobulins showed normal IgA slightly elevated IgG and IgE and a high concentration of IgM Agarose and immunoelectrophoresis revealed that the patient had a monoclonal IgMκ protein Bone marrow aspiration showed a normal plasma cell count Urine analysis revealed periodical microscopic hematuria and a slight albuminuria Traces of Bence Jones protein in urine have been repeatedly demonstrated during the last two years

Histology of biopsies from diseased skin showed a moderate perivascular infiltration of lymphocytes and polymorphonuclear cells The majority of these cells were neutrophils but some eosinophils could also be demonstrated

Immunofluorescence studies of biopsy specimens from the skin lesions showed diffuse deposits of IgM in the dermus and granular deposits of the same immunoglobulin in the junction between dermis and epidermis

The arthralgias improved remarkably with prednisone 25 mg daily but the drug had no effect on the skin lesions A trial with Imurel® and dapsone had no effect Plasmapheresis was also tried 20 l plasma was exchanged

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*Abbreviations* FITC=fluorescein isothiocyanate PBS=phosphate buffered saline PHA=phytohemagglutinin PWM=pokeweed mitogen ELISA=enzyme linked immunosorbent assay OD=optical density $(Fc)_5\mu$ $F(ab)_n$= IgM molecules with different number of F(ab) fragments removed protein A=protein A from Staphylococcus aureus

during a 14-day period by means of a Haemonetix cell separator but had no effect on the skin lesions

*Isolation and purification of the monoclonal protein* The monoclonal IgMκ protein from the patient (OJL) was isolated by means of euglobulin precipitation with 0 1% boric acid (I=0 1) in a dilution of 1/20 (V/V) The precipitate was dissolved in 0 05 M Tris/HCl buffer pH 7 6 and dialyzed against the same buffer before further purification by gel filtration on a Sephadex G-200 column The IgM-containing fraction was rechromatographed on the same column and purified further on immunosorbent columns containing 1) protein A 2) rabbit antihuman α chain antibodies 3) rabbit antihuman λ light chain antibodies In each case the protein was coupled to Sepharose 4B (Pharmacia) by the CNBr method (13)

*Production of Fab fragments from the M component* The OJL IgM protein isolated as described above was further degraded by pepsin digestion at pH 8 4 for 30 min at 56 C (14) The degradation products were separated on a Sephadex G 200 column eluted with 0 05 M Tris/HCl buffer pH 7 6 The gel filtration gave two protein peaks one void fraction corresponding to $(Fc)5\mu$ F(ab) and one corresponding to F(ab) fragments in molecular weight

*Antisera* Antiserum against the IgMκ protein was raised in a rabbit by repeated immunizations with 0 1 mg of the M component in complete Freund s adjuvant The antiserum was then absorbed by subjecting it to immunosorbent columns with the following antigens coupled to Sepharose 4B light chains of pooled IgG two monoclonal IgM proteins pooled IgG (Kabi Sweden) and normal human serum

Class specific antisera as well as an antiserum against $F(ab')_2$ fragments of human IgG were raised in rabbits and conjugated with fluorescein isothiocyanate (FITC) as described earlier (10) Antibodies against human $F(ab')_2$ fragments were also conjugated with tetramethyl rhodamine isothiocyanate (10) IgG was isolated from the idiotype specific antiserum by ion exchange chromatography and subsequently pepsin-digested as described earlier (12) The $F(ab')_2$ fragments were isolated after gel filtration on a Sephadex G 200 column under neutral conditions (12) FITC-conjugated goat antirabbit immunoglobulin antiserum was purchased from Behringwerke Marburg Lahn W Germany

*Isolation and characterization of peripheral blood lymphocytes* Lymphocytes were separated by the Isopaque Ficoll gradient centrifugation technique (3) Membrane bound Ig determined by the immunofluorescence technique was used as a marker for B lymphocytes (8) T lymphocytes have receptors for sheep erythrocytes and were detected with a rosette technique as described earlier (8) and lymphocytes with receptors for the Fc part of IgG were detected using human O Rh+ red cells sensitized with anti Rh Ripley antibodies (8) Isolation of T lymphocytes was performed in three ways 1) by passing lymphocytes through a glass column containing nylon wool fibres (Fenwal Lab Morton Grove Ill ) as described earlier (8) 2) by sedimentation of E rosettes formed with sheep red blood cells treated with 2 aminoethylisothiouronium bromide (Sigma Chemical Co St Louis Mo ) as described elsewhere (5) and 3) by passing lymphocytes through immunosorbent columns to which rabbit antihuman IgG antibodies were coupled as described elsewhere (1) Enriched B lymphocytes were eluted from the gel by gentle stirring of the gel with a Pasteur pipette as described elsewhere (1) The lymphocytes isolated in these three ways were then incubated for 1 hour at 37°C 5% $CO_2$ and 100% humidity in RPMI 1640 (Grand Island Biological Co ) with 15% heat inactivated fetal calf serum $10^{-5}$ M mercaptoethanol and penicillin/streptomycin

*Trypsin treatment of B and T lymphocytes* Equal volumes of trypsin (Sigma Chemical Co ) dissolved in phosphate buffer pH 7 6 (5 mg/ml) and T and B lymphocytes ($2\times10^7$/ml) and Hanks balanced salt solution were incubated at 37°C for 15 min The reaction was stopped with ice-cold RPMI 1640 and the cells were washed twice

*Immunofluorescence staining and microscopy* Direct and indirect immunofluorescence staining was performed as outlined before (16) Briefly direct immunofluorescence staining was performed by incubating $5\times10^5$ peripheral blood lymphocytes with the various FITC conjugated antisera for 20 min at 4 C with subsequent washing Indirect immunofluorescence staining was performed by first incubating the lymphocytes with anti idiotype antibodies for 20 min at 4 C After washing FITC-conjugated goat antirabbit antiserum was added and left to react for 20 min at 4 C before washing Double immunofluorescence staining was performed as previously described (10)

Skin tissue sections of 4 μm from the patient s and two normal skin biopsy specimens were cut in a cryostat and air-dried without fixation The sections were first washed in phosphate buffered saline (PBS) pH 7 4 for 10 min and then incubated with FITC-conjugated anti class antisera and the anti idiotype antiserum for 30 min in moist chamber After washing the section that had reacted with the anti idiotype antiserum was further stained by adding FITC-conjugated goat antirabbit antiserum for another 30 min in the same way as outlined above Normal skin sections were incubated with the M component and its Fab fragments for 40 min at room temperature After washing the sections were stained with FITC-conjugated anti μ and anti $F(ab')_2$ antisera

*Stimulation of lymphocytes with unspecific mitogens* Peripheral blood lymphocytes from the patient were stimulated with phytohemagglutinin (PHA) 1 50 (Wellcome Research Laboratories Beckenham England) and with pokeweed mitogen (PWM) 1 4 (Grand Island Biological Co Grand Island New York) as described earlier (17) Briefly 50 μl of lymphocytes (1 mill/ml) 50 μl medium RPMI 1640 (Grand Island Biological Co ) plus 20 μl of PHA or PWM were mixed in microtitre plates The lymphocytes were incubated at 37°C 5% $CO_2$ and 100% humidity for 4 days $^3H$ thymidine was added 16 hours before harvesting the cultures The lymphocyte transformation was calculated from $^3H$ thymidine incorporation expressed as disintegrations per min in a liquid scintillation counter (17)

*Enzyme linked immunosorbent assay (ELISA) for determination of anti idiotypic specificity of the antiserum* (2 6 7) The test was performed in disposable polystyrene tubes (NUNC N1007 Denmark) All incubations were

Table I *Test for anti idiotypic specificity of absorbed antiserum against OJL IgM and other monoclonal IgM proteins by indirect ELISA method*

The antiserum was used in a dilution of 1/4 000
Control experiments in which tubes with the same coats were incubated with conjugate gave an $OD_{400}$/100 min of less than 0 1

| Coat (2 µg/ml) | $OD_{400}$/100 min |
|---|---|
| Pooled IgG (Kabi) | 0 04 |
| OJL IgMκ | 1 93 |
| Tor IgMκ | 0 09 |
| Wil IgMκ | 0 11 |
| Sche IgMκ | 0 02 |
| West IgAκ | 0 01 |
| Eng IgAλ | 0 01 |
| Buffer | 0 11 |

performed at room temperature The tubes were coated by incubation with 1 0 ml of a solution of human immunoglobulin in a coating buffer consisting of 0 05 M sodium carbonate buffer pH 9 6 with 0 05% $NaN_3$ for 2 hours After washing three times with 0 9% NaCl with 0 05% Tween 20 the tubes were incubated for 6–8 hours with 1 0 ml of antiserum in PBS with 0 05% Tween 20 and 0 05% $NaN_3$ After this incubation the tubes were washed as described above and incubated with 1 0 ml of swine anti-rabbit IgG antibodies conjugated with alkaline phosphatase (Orion Finland) The conjugate was used in a dilution of 1/500 with PBS containing 0 05% Tween 20 and 0 05% $NaN_3$ The incubation time was 10–15 hours

After subsequent washing as described above 1 0 ml of the substrate solution p-nitrophenylphosphate (1 mg/ml) in 1 M diethanolamine HCl buffer pH 9 8 was added to the tubes and the enzymatic reaction was stopped by adding 100 µl of 5 M NaOH to the tubes The reaction was stopped before the solution had an optical density (OD) = 1 at 400 nm and the $OD_{400}$ measured was referred to a standard time 100 min assuming a linear reaction rate (7)

Inhibition experiments were performed as described above with the exception that the inhibiting protein was added to the tubes at the same time as the antiserum (after the tubes had been coated) This step had also a total incubation volume of 1 ml

## RESULTS

### *Agarose and immunoelectrophoresis*

By means of agarose electrophoresis a band corresponding to the application line was seen Immunoelectrophoresis revealed that the band represented a monoclonal IgMκ protein

### *Specificity controls of the anti idiotype antiserum*

The anti idiotype antiserum was absorbed on immunosorbent columns to which monoclonal IgM



*Fig 1* Granular deposits of idiotype positive material along the dermal/epidermal junction and in the dermis

proteins and pooled normal human IgG were coupled until it reacted only with the immunizing protein OJL but neither with other monoclonal IgM proteins (Tor Wil Sche) nor monoclonal IgA proteins or pooled IgG in the ELISA technique (Table I) The specificity of the anti idiotype antiserum was also assessed in inhibition experiments using the ELISA method OJL whole IgM and OJL IgM Fab fragments inhibited the idiotypic reaction totally whereas other monoclonal proteins had no effect (Table II)

### *Markers and stimulability of the patient s peripheral blood lymphocytes*

The different markers of the peripheral blood lymphocytes are listed in Table III 15% of the

Table II *Test for anti idiotypic specificity of absorbed antiserum against OJL IgM by ELISA inhibition method*

The antiserum was used in a dilution of 1/5 000
The tubes were coated with 0 2 µg/ml of OJL IgMκ protein

| Coating protein | Inhibitor (µg/ml) | $OD_{400}$/100 min |
|---|---|---|
| OJL IgMκ | OJL IgMκ 2 | 0 15 |
| OJL IgMκ | OJL IgMκ 0 2 | 0 16 |
| OJL IgMκ | OJL IgMκ 0 02 | 0 78 |
| OJL IgMκ | OJL F(ab) 2 | 0 14 |
| OJL IgMκ | OJL F(ab) 0 2 | 0 32 |
| OJL IgMκ | OJL F(ab) 0 02 | 0 99 |
| OJL IgMκ | Pal IgGκ 2 | 1 88 |
| OJL IgMκ | JAV IgGκ 2 | 1 81 |
| OJL IgMκ | TIL IgGκ 2 | 1 85 |
| OJL IgMκ | West IgAκ 2 | 1 86 |
| OJL IgMκ | Buffer | 1 79 |
| Buffer | Buffer | 0 11 |

Table III *Percentages of lymphocytes staining with anti F(ab )₂, anti IgM anti IgD and anti idiotype antisera and percentages of EA and E rosette forming cells (RFC)*

| | Anti F(ab )₂ | Anti IgM | Anti IgD | Anti idiotype | EA RFC | E RFC |
|---|---|---|---|---|---|---|
| Unfractionated lymphocytes | 25 | 20 | 15 | 15 | 11 | 61 |
| B lymphocytes | 40* | NT | NT | 12 | NT | NT |
| T lymphocytes | 1 | NT | NT | 12 | 9 | 92 |

Less than 1% of the lymphocytes stained positively with anti IgG anti IgA and anti IgE Peripheral blood lymphocytes from two normal blood donors did not stain with the anti idiotype antiserum
8% of the peripheral blood lymphocytes stained both with the anti idiotype antiserum and an anti F(ab )₂ antiserum
* B cells enriched by E RFC depletion
NT – not tested

peripheral blood lymphocytes stained with the anti idiotype antiserum Double staining experiments revealed that 8% of the B cells stained positively with anti idiotype antiserum Furthermore 12% of the T lymphocytes isolated in the three ways de scribed in Materials and Methods were also positive with the anti idiotype antiserum Less than 1% of these T lymphocytes stained positively with FITC-conjugated anti F(ab )₂ antiserum Staining of the lymphocytes with anti idiotype antiserum af ter incubation at 37°C for 1 hour with subsequent washing gave the same results Trypsin treatment of the B and T lymphocytes removed the mem brane bound Ig and the idiotypic markers since staining with anti immunoglobulin antiserum and anti idiotypic antibodies gave negative results Af ter 16 hours incubation both the Ig and the idiotype positive markers on the lymphocytes reappeared (Table IV) The PHA and PWM re sponses of the peripheral lymphocytes were nor mal

*Demonstration of idiotype positive molecules in the skin*

Staining of skin tissue sections with anti idiotype anti F(ab )₂ and anti Ig class antisera revealed that IgM molecules positive for the OJL IgM idiotype were bound to the dermal/epidermal junction of th diseased skin (Fig 1) Idiotype positive materi was also observed in the vessel walls of the dermis The idiotype positive material was demonstrated i diseased skin only while unaffected skin was nega tive Incubation of skin sections from two othe normal humans with the M component and its Fa fragments and with subsequent staining with anti and anti F(ab )₂ antisera did not reveal any bindin of the IgM molecules

## DISCUSSION

In the present study an anti idiotype antiserun against a monoclonal IgM protein reacted both witl peripheral blood B and T lymphocytes of the pa tient This means that membrane bound molecule: on circulating lymphocytes in this patient have simi lar antigen binding sites as the monoclonal IgM protein in the patient s serum used for immuniza tion Similar results have been observed in othe human systems too (2 3 4) but this is the firs report on common idiotypes on B and T lympho cytes in a patient with macroglobulinemia The idiotype positive molecules demonstrated on the

Table IV *Percentages of lymphocytes staining positively with anti F(ab )₂ and anti idiotype antibodies before and after trypsinization of the lymphocytes*

| | Immediately after trypsinization | | 16 hours after trypsinization | |
|---|---|---|---|---|
| | Anti F(ab )₂ | Anti idiotype | Anti F(ab )₂ | Anti idiotype |
| B lymphocytes | <1 | <1 | 18 | 10 |
| T lymphocytes | <1 | <1 | <1 | 14 |

lymphocytes seemed not to be passively absorbed since they could not be removed by incubation at 37°C for 1 hour (18) On the contrary the idiotype positive molecules were actively synthesized by the lymphocytes since the molecules reappeared on the B and T lymphocytes in culture after removal of the idiotype positive molecules from the cells by trypsin treatment

Our patient had an urticaria like skin disease and IgM positive material bound to the dermal/epidermal junction was demonstrated in diseased skin tissue sections Immunofluorescence studies with anti idiotype antibodies revealed that these IgM molecules had the same idiotypes as the monoclonal serum component No IgM- or idiotype positive material could be demonstrated in sections from unaffected skin Furthermore no binding of the M component to skin structures could be demonstrated in biopsies from other patients It is not known whether the M component is responsible for the skin disease or whether its presence is only secondary to vasodilatation The granular deposits of the idiotype positive IgM molecules to the dermal/epidermal junction area indicate a specific binding of these molecules No IgG or IgA could be demonstrated in the same area and this contradicts the hypothesis that the IgM present is caused by passive exudation of the M component secondary to vasodilatation Repeated plasma exchange transfusions did not lead to any improvement of the skin disease but there could always be enough of the M component left in the serum and the skin to cause the urticarial reaction The antibody specificity of the M component is unknown but it reacts most likely with an antigen present only in the diseased skin of the patient This antigen is not present or demasked in the undiseased skin of the patient or in skin sections from other humans The unknown antigen might represent an autoantigen (i e altered self)

The fact that the lymphocytes and the circulating and skin bound molecules of the M component have the same idiotypes indicates that they all might be directed against one and the same unknown antigen This is also indicated by the fact that in addition to skin bound IgM molecules with the same idiotypic determinants as those of the M component you find lymphocytes infiltrating the diseased skin tissues The idiotypes and antibody specificities of the membrane bound molecules of these lymphocytes are not known and are hard to reveal but they may also be directed against the same antigen Thus both humoral and cellular immunity could play an important role in the pathogenesis of the urticarial skin disease of this patient The circulating B lymphocytes are probably ancestors of the major plasma cells that are producing the M component The role of the idiotype positive T cells is not yet known but they may be regulator cells (helper and/or suppressor T cells) or effector cells playing an important role in the immune response to an unknown antigen

We used a highly specific anti idiotype antiserum to localize a certain antibody population in the skin tissue of the patient Furthermore it was used to demonstrate membrane bound molecules on circulating B and T cells with the same idiotypes (i e antigen binding site) as the IgM molecules of the M component

The use of anti idiotypic antibodies in medicine is probably at its starting point These antibodies are as outlined before highly specific for certain circulating and cell bound antibody molecules Thus anti idiotypic antibodies might be used in the future to destroy harmful lymphoid cells like leukemic cells and myeloma cells through cytotoxic mechanisms involving complement in the body or radioactive and cytostatic drugs coupled to the anti idiotypic antibodies Anti idiotypic antibodies may also be used in order to control the effect of treatment since they can trace small amounts of certain antibody populations and small numbers of neoplastic cells

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## REFERENCES


1 Antoine J C Ternynck T Rodrigot M & Avrameas S Lymphoid cell fractionation on magnetic polyacrylamide agarose beads Immunochemistry 15 443 1978
2 Avrameas S Coupling of enzymes to proteins with glutaraldehyde Use of the conjugates for the detection of antigens and antibodies Immunochemistry 6 43 1969
3 Bøyum A Separation of leucocytes from blood and bone marrow Scand J Clin Lab Invest (Suppl) 97 1968
4 Capra J D & Kehoe J M Hypervariable regions idiotypy and antibody-combining site Adv Immunol 20 1 1975

5 Dobloug J H Førre Ø Natvig J B & Michaelsen T E Demonstration of rheumatoid factor idiotypic antigens on peripheral blood B and T lymphocytes from patients with rheumatoid arthritis Scand J Immunol 9 273 1979
6 Engvall E & Perlmann P Enzyme linked immunosorbent assay (ELISA) Quantitative assay of immunoglobulin G Immunochemistry 8 871 1971
7 — Enzyme linked immunosorbent assay (ELISA) III Quantitation of specific antibodies by enzyme labelled anti-immunoglobulin in antigen coated tubes J Immunol 109 129 1972
8 Frøland S S & Natvig J B Identification of three different human lymphocyte populations by surface markers in T and B lymphocytes in humans Transplant Rev 16 114 1973
9 Fu S M Winchester R J Feizi T Waltzer P D & Kunkel H G Idiotypic specificity of surface immunoglobulin and the maturation of leukemic bone marrow-derived lymphocytes Proc Natl Acad Sci USA 71 4487 1974
10 Førre Ø et al A study of the variable heavy chain ($V_H$) region of membrane bound Ig on human chronic leukemic lymphocytes J Immunol 118 (5) 1513 1977
11 Holm G Mellstedt H Petterson D & Biberfeld P Idiotypic immunoglobulin structures on blood lymphocytes in human plasma cell myeloma. Immunol Rev 34 39 1977
12 Lea T Førre Ø Michaelsen T E & Natvig J B Shared idiotypes on human peripheral blood B and T lymphocytes J Immunol 122 2413 1979
13 March S C Parikh I & Cuatrecasas P A simplified method for cyanogen bromide activation of agarose for affinity chromatography Anal Biochem 60 149 1974
14 Plaut A G & Tomasi T B Jr Immunoglobulin M Pentameric Fcμ fragments released by trypsin at higher temperatures Proc Natl Acad Sci USA 65 317 1970
15 Preud'homme J L Klein M Labaum S & Seligmann M Idiotype bearing and antigen binding receptors produced by blood T lymphocytes in a case of human myeloma. Eur J Immunol 7 840 1977
16 Salsano F Frøland S S Natvig J B & Michaelsen T E Same idiotype of B-lymphocyte membrane IgD and IgM Formal evidence for monoclonality of chronic lymphocytic leukaemia cells Scand J Immunol 3 841 1974
17 Thoresen A B Nousiainen H Hirschberg H & Thorsby E Activation of human lymphocytes by allogenic cells (MLC) antigens and mitogens in vitro Proc Australas Tissue Typing Workshop 1977
18 Winchester R J Fu S M Hoffman T & Kunkel H G IgG on lymphocyte surfaces technical problems and the significance of a third cell population J Immunol 114 (4) 1210 1975

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# Blood Coagulation Studies in 45 Patients with Ischemic Cerebrovascular Disease and 44 Patients with Venous Thromboembolic Disease

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ABSTRACT Forty five patients with ischemic cerebrovascular disease (ICD) and 44 with deep venous thrombosis (DVT) and/or pulmonary embolism (PE) have been investigated in a non-active state of the disease with VIIIR Ag plasminogen activator before and after stasis antiplasmin antithrombin (activity, antigen activity/antigen ratio) and spontaneous platelet aggregation Control groups of 20 respectively 80 healthy females and males were used in the study VIIIR Ag was elevated in the group with deep venous thromboembolic disease compared with the ICD group and a control group VIIIR Ag in the ICD group was elevated compared with the control group Plasminogen activator determined before and after stasis was lowered in the two diseased groups There was no statistically significant difference in any of the blood coagulation variables between patients on or off coumarol treatment The patients on coumarol were however not reinvestigated when this medication had been withdrawn Antithrombin levels below the reference interval of the control group of 80 blood donors were found in 11 4% of the patients with DVT/PE while no patient in the ICD group had low antithrombin values

*Key words* VIIIR Ag plasminogen activator antiplasmin antithrombin III spontaneous platelet aggregation

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A standardized set of coagulation laboratory variables was introduced in 1976 at the Department of Blood Coagulation Disorders in Stockholm to provide a broad basis for evaluation of risk factors in thromboembolic diseases expressed by blood coagulation tests This basis was intended to cover disturbances in (*a*) fibrinolytic activity in the plasma (9 13) (*b*) aggregation of platelets (4) (*c*) blood coagulation (2 7 10 15)

Blood coagulation studies on 89 patients with four main diagnoses of venous and arterial thromboembolic disorders are presented in this study

## PATIENTS AND METHODS

All patients with the diagnosis of thromboembolic disease admitted to (and accepted for) blood coagulation investigations between Nov 1976 and Sept 1977 are the basis of this study We investigated all patients by means of eight blood coagulation variables (see below) The median time from the onset of the disease to blood sampling for coagulation tests was 5 6 months A total of 53 females and 68 males were investigated during the study period All patient records were checked retrospectively in order to get information on the diagnostic criteria used by the admitting department

Only patients who could be classified as having transient ischemic attack(s) (TIA) cerebral infarctions (CI) and deep venous thrombosis (DVT) and/or pulmonary embolism (PE) including recurrent DVT/PE were accepted for the study Five females and six males were excluded as they belonged to other diagnostic groups Five patients in the ischemic cerebrovascular disease (ICD) group and 16 in the DVT/PE group were excluded as the diagnoses were based on clinical symptoms or were not sufficiently documented in the patient records The diagnostic groups with median age and median interval between the latest expression of the disease and blood sampling are presented in Table I

### *Diagnostic groups*

*Ischemic cerebrovascular disease (TIA CI)* All 45 patients in this group were admitted from and diagnosed by neurological specialists and were investigated with combinations of computed tomography cerebral scintigraphy aortic arch angiography CSF spectorphotometric investigations etc

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*Abbreviations* ICD = ischemic cerebrovascular disease DVT = deep venous thrombosis PE = pulmonary embolism TIA = transient ischemic attack CI = cerebral infarction AT sub = antithrombin substrate AT = antithrombin antigen

Table I *Some data on the patient and control groups*

| | *n* | Female/male ratio | Median age (y) | Median time from disease to blood test (mo) |
|---|---|---|---|---|
| TIA | 16 | 7/9 | 50 | 4 4 |
| CI | 29 | 11/18 | 48 | 5 2 |
| DVT/PE | 14 | 7/7 | 37 | 7 5 |
| Recurrent DVT/PE | 30 | 11/19 | 40 | 5 6 |
| Control I | 20 | 10/10 | 39 | |
| Control II | 80 | 40/40 | 40 | |
| Significance of differences between the groups | | | n.s | n s |

*Venous thromboembolic disease (DVT/PE recurrent DVT/PE)* All 44 patients in this group were investigated with phlebography and/or pulmonary scintigraphy The group comprised patients who had had one episode of DVT/PE or at least two episodes (recurrent DVT/PE)

Of the 89 patients investigated in this study 33 had various chronic diseases and six of the females used contraceptive drugs when falling ill (Table II)

*Blood coagulation tests*

All assays except 2, 3 and 8 (below) were standardized against an internal plasma standard from 18-23 healthy males

1 VIIIR.Ag Zimmerman et al (15) Reference interval 28-194%

2-3 Plasminogen activator before and after venous occlusion. Noren et al (9) Reference interval 0 05-0 33 and 0.27-0 63 arb U respectively

4 Antiplasmin with chromogenic peptide substrate Teger Nilsson et al. (13) Reference interval 90-130%

5 Antithrombin activity with chromogenic peptide substrate (AT sub) Ødegaard et al (10) Reference interval 83-128%

6 Antithrombin antigen (AT Ag) with immunological method Fagerhol and Abildgaard (2) Mancini et al (7) Reference interval 78-123%

7 The ratio between antithrombin levels in the diagnostic patient groups (substrate/antigen) was also analysed.

8 Spontaneous platelet aggregation. Holdrinet et al (4) modified according to Mettinger et al (8) Reference interval <25%

*Control groups*

Ten females and 10 males 21-61 years of age (healthy blood donors) served as control group for statistical comparison with the diagnostic patient groups Reference values for VIIIR.Ag, antiplasmin and antithrombin were obtained from a second control group of 40 females and 40 males 21-61 years of age (healthy blood donors) (14)

*Medication* (Table III)

Hormone preparations were withdrawn 3 months and aspirin at least 10 days before blood coagulation samples were drawn. Patients on coumarol remained on their medication

Table II *Patients with chronic diseases and on oral contraceptive drugs*

| Patient group | Neoplastic disease | Liver disease incl alcoholism | Hypertension | Contraceptive drugs[a] | Other chronic diseases[b] |
|---|---|---|---|---|---|
| TIA | | | 3 | 3 | Diabetes mellitus<br>Hyperlipemia |
| CI | | | 6 | | Lues<br>Diabetes mellitus<br>Hyperlipemia |
| DVT/PE | 1 | 1 | 2 | 3 | Splenomegaly |
| Recurrent DVT/PE | 3 | 3 | 3 | | Epilepsy<br>Bronchitis chron.<br>MB Crohn<br>Hypothyrosis<br>Hyperlipemia |

[a] Withdrawn at the onset of thromboembolic disease

[b] One case each.

Table III *Patients on oral anticoagulants*

| Patient group | Waran/AP | Aspirin | No medication |
|---|---|---|---|
| ICD | 7 | 22 | 16 |
| DVT/PE | 34 | 0 | 10 |

*Statistical methods*

Differences between three or more independent diagnostic groups were analysed by Kruskal Wallis analysis of variance Differences between two independent diagnostic groups or combinations of groups were analysed by Mann Whitney *U* test Differences between independent means of gropus ($n \leq 10$) were analysed by the *t* test Spearman rank correlation test was used for correlation analyses

## RESULTS

The median values ranges and analysis of variance are presented in Table IV More details concerning the diagnostic groups are shown in Table I No significant differences between females and males were found in the diagnostic and the control groups with respect to blood coagulation variables why both females and males are included in all these groups No significant differences were found between the diagnostic groups with respect to age or time from the latest expression of the disease to sampling of blood (Table I) The ICD patients as a total group were however older than DVT/PE patients

The one way analysis of variance showed a statistically signficant difference between the four patient groups and the control group of 20 normals in VIIIR Ag and plasminogen activator before and after stasis (Table IV) Further statistical analysis of possible causes of variation (Table V) showed that VIIIR Ag was elevated in the total DVT/PE group compared with the control group and the total ICD group VIIIR Ag was also elevated in the ICD group when compared with the control group In the total groups of patients with DVT/PE or ICD there was no significant difference in any of the variables between patients with and without coumarol medication

The control group had a higher level of plasminogen activator before and after stasis than the disease groups among which no statistically significant differences were found A strong to moderate correlation between the two antithrombin methods existed in the two disease groups and in the control group A moderate correlation between VIIIR Ag and age was found among patients with DVT/PE Other slight but significant correlations between some of the laboratory variables are listed in Table VI Five patients all in the DVT/PE group had antithrombin values below the reference intervals (14) (AT sub <83% or AT-Ag <78%)

## DISCUSSION

The results of several studies on blood coagulation variables as risk factors for thromboembolic disorders are contradictory This is probably due to heterogeneity in the investigated patient groups with respect to age medication type of thromboembolic disorder coexisting malignant or reactive disorders etc

Hedner and Nilsson (3) found that antithrombin measured by two-stage clotting method and immunological method was in general normal or elevated in patients with recurrent venous thrombosis acute myocardial infarction malignant disease and during oral contraceptive medication Essentially the same results were achieved by Okuno and Crockatt (11) in antithrombin measurements by two-stage clotting method In the present study 11 4% (5 patients) with DVT/PE had however low antithrombin values a figure that agrees with those found by Collins et al (1) and Lechner et al (6) 17 6 and 9 3% respectively In the latter study antithrombin values increased during oral anticoagulant treatment Our patients on such medication were not studied after withdrawal of the treatment

Among patients with ischemic heart disease Stormorken and Eriksen (12) demonstrated low antithrombin values (chromogenic substrate method) in angiopositive patients with angina pectoris Persons with blood group A had lower antithrombin values than those with blood group O Mettinger et al (8) found somewhat higher antithrombin values (immunological method) in patients with ICD than in a healthy control group The mean value in our patients with ICD was around that of the controls In conformity with the observations by Mettinger et al we found no statistically significant differences in values of spontaneous platelet aggregation between the diagnostic and the control groups

Isacsson and Nilsson (5) reported a relation between defective release of plasminogen activator in

Table I. *Some data on the patient and control groups*

| | *n* | Female/male ratio | Median age (y ) | Median time from disease to blood test (mo ) |
|---|---|---|---|---|
| TIA | 16 | 7/9 | 50 | 4 4 |
| CI | 29 | 11/18 | 48 | 5 7 |
| DVT/PE | 14 | 7/7 | 37 | 7.5 |
| Recurrent DVT PE | 30 | 11/19 | 40 | 5 6 |
| Control I | 20 | 10/10 | 39 | |
| Control II | 80 | 40/40 | 40 | |
| Significance of differences between the groups | | | n s | n.s |

*Venous thromboembolic disease (DVT PE, recurrent DVT PE)* All 44 patients in this group were investigated with phlebography and/or pulmonary scintigraphy The group comprised patients who had had one episode of DVT PE or at least two episodes (recurrent DVT/PE)

Of the 89 patients investigated in this study 33 had various chronic diseases and six of the females used contraceptive drugs when falling ill (Table II)

*Blood coagulation tests*

All assays except 2, 3 and 8 (below) were standardized against an internal plasma standard from 18–23 healthy males.

1 VIIIR.Ag Zimmerman et al. (15) Reference interval 28–194%

2 3 Plasminogen activator before and after venous occlusion. Noren et al. (9) Reference interval 0 05–0.33 and 0.27–0.63 arb U respectively

4 Antiplasmin with chromogenic peptide substrate Teger Nilsson et al. (13) Reference interval 90–130%

5 Antithrombin activity with chromogenic peptide substrate (AT-subr) Odegaard et al. (10) Reference interval 83–128%

6 Antithrombin antigen (AT Ag) with immunological method. Fagerhol and Abildgaard (7) Mancini et al. (7) Reference interval 8–123%

7 The ratio between antithrombin levels in the diagnostic patient groups (substrate/antigen) was also analysed.

8 Spontaneous platelet aggregation. Holdrinet et al (4) modified according to Mettinger et al. (8) Reference interval <25%

*Control groups*

Ten females and 10 males 21–61 years of age (healthy blood donors) served as control group for statistical comparison with the diagnostic patient groups Reference values for VIIIR.Ag, antiplasmin and antithrombin were obtained from a second control group of 40 females and 40 males 21–61 years of age (healthy blood donors) (14)

*Medication* (Table III)

Hormone preparations were withdrawn 3 months and aspirin at least 10 days before blood coagulation samples were drawn. Patients on coumarol remained on their medication.

Table II *Patients with chronic diseases and on oral contraceptive drugs*

| Patient group | Neoplastic disease | Liver disease incl alcoholism | Hypertension | Contraceptive drugs[a] | Other chronic diseases[b] |
|---|---|---|---|---|---|
| TIA | | | 3 | 3 | Diabetes mellitus<br>Hyperlipemia |
| CI | | | 6 | | Lues<br>Diabetes mellitus<br>Hyperlipemia |
| DVT/PE | 1 | 1 | 2 | 3 | Splenomegaly |
| Recurrent DVT/PE | 3 | 3 | 3 | | Epilepsy<br>Bronchitis chron.<br>MB Crohn<br>Hypothyrosis<br>Hyperlipemia |

[a] Withdrawn at the onset of thromboembolic disease

[b] One case each.

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# The Preleukemic Syndrome

## I Clinical and Hematological Findings

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**ABSTRACT** A long term prospective study including 151 patients with preleukemia was performed in 1958–79 The series comprised 78 women and 73 men with a mean age of 79 and 72 years, respectively Acute leukemia was the cause of death in 35 patients, 61 died of infections and/or hemorrhage or of unrelated causes The mean interval between the initial diagnosis and death from acute non lymphocytic leukemia was 36 months (range 2–121) Pitfalls in diagnosis are extensively discussed The most striking was the advanced age of the patients The presence of pancytopenia at the time of diagnosis was not predictive for subsequent blastic transformation

*Key words* preleukemic syndrome advanced age long term follow up

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In a variety of retrospective studies of patients with acute myeloid leukemia it has been shown that hematological abnormalities preceding overt leukemia can be observed In 1973 these studies were reviewed by Saarni and Linman (21) and the characteristics of the hematological abnormalities were compared with the clinical findings in 34 patients who developed acute non lymphocytic leukemia (20) These characteristics were considered to be sufficiently uniform to permit prospective studies (9 13 18) The term preleukemia was introduced by Block et al in 1953 (3) Preleukemic syndrome instead of preleukemia has been proposed by Linman (14 15) as a useful designation for the hematological abnormalities which may precede the development of acute non lymphocytic leukemia This designation includes a number of disorders which have been signified under different headings Di Guglielmo syndrome chronic erythroleukemia chronic myelomonocytic leukemia etc The classification of these different hematological pictures under one heading appears to be justified because there is a considerable overlap between them The hematological picture may change during the course of the disease Moreover circumstantial evidence has been presented that the syndrome always involves all myeloid cell lines but never one single cell line (13) They are therefore diseases of a *multipotential marrow stem cell (13)* The term myeloid dysplasia has been proposed mostly for psychological reasons as an alternative designation (1)

The purpose of this paper is to report our experience during more than 20 years with preleukemia Special attention has been paid to diagnostic problems and the natural course of the disease

## PATIFNTS AND METHODS

The study of patients with suspected preleukemia was initiated in 1958 and is still continuing During 1958–79 151 patients were included They had been referred by their general practitioners to the Department of Hematology Municipal Hospital the Hague for evaluation of unexplained anemia leucopenia or hemorrhagic phenomena or by other departments where a hematological disorder was found accidentally during routine examination

Initial work up of the disease consisted of a complete history and physical examination In addition laboratory tests were carried out including a complete blood count peripheral blood smears bone marrow studies protein electrophoresis vitamin $B_{12}$ folic acid serum iron iron binding capacity and when indicated the LE cell test The bone marrow aspirates were stained with both Wright s stain for morphology and Prussian blue for demonstration of iron Ringed sideroblasts were defined as normoblasts containing 8 or more granules around the nucleus In order to compare these results with a recent study by Hast (10) a more precise differentiation was made between ringed

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normal rise of platelets during the first treatment with cyanocobolamine did not occur in this patient and the original neutropenia persisted After 4 years a preleukemic syndrome developed and cytogenetic studies showed an abnormal clone This was the only case with preleukemia observed among about 400 patients with pernicious anemia during the same period This finding suggests that there is no increased incidence of preleukemia in pernicious anemia and seems to be in accordance with the findings of a previous study regarding acute leukemia and pernicious anemia (2)

Most of our patients did not receive cancer chemotherapy and/or immunodepressive drugs In those who were treated the results were very poor as in other observations Two of our patients died of tuberculosis during treatment with prednisone The diagnosis of preleukemia was made long before initiation of this treatment They were treated with blood transfusions alone for a considerable period during which they showed no signs of tuberculosis

The impression exists that the patients with preleukemia (myelodysplasia) reported in this study are suffering from a heterogeneous group of disorders Attempts to define separate entities like refractory sideroblastic anemia smouldering leukemia or subacute myeloid leukemia have however been unsatisfactory because of the great overlap between these syndromes Moreover it can be difficult to differentiate cases with myelodysplasia showing a progressive course and terminating as acute myeloid leukemia from cases presenting as acute myeloid leukemia in which the possibility of a preleukemic phase cannot be excluded because of insufficient data This differentiation is important because the results of treatment of patients with acute myeloid leukemia after a preleukemic state are very poor

In elderly patients the hematologic abnormalities of the preleukemic state may be undetected for a long time and general aspecific symptoms are often considered to be due to other illnesses or to advanced age In view of the surprising frequency of myelodysplastic changes in the elderly regular control of the blood seems advisable In this way a preleukemic state can nearly always be diagnosed or at least seriously suspected Knowledge of the existence of myelodysplastic changes is important for the management because these patients may need extra supportive care at operations after accidents and in case of infection

Other important aspects of the preleukemic syndrome are the preleukemic changes observed in patients treated with cytostatics or irradiation not only because they can develop into acute myeloid leukemia but also because they can interfere with further cytostatic treatment Moreover a preleukemic syndrome can be present prior to cytostatic drug treatment e g in patients with multiple myeloma (19) Exposure to myelotoxic treatment like irradiation and cytostatic or anti inflammatory agents can precede myelodysplasia by many years It is therefore to be expected that their influence in patients with preleukemia may be more frequent than is apparent from the case histories Accurate satisfactory parameters predictive for the future course of the preleukemic syndrome (survival time acute blastic transformation) have not yet been determined The presence of an initial pancytopenia has been considered to be predictiv for subsequent acute transformation (17) which was not confirmed in our patients Only 4 out of our 35 patients with blastic transformation had initial pancytopenia

Recent studies suggest that in vitro marrow myeloid clonal growth can be of prognostic significance Markedly diminished values of initial granulocyte monocyte colony forming cells were found to be predictive for a significantly decreased probability of survival (9) Long term prospective studies now in progress (14) will possibly help to solve some of the problems encountered in the management of patients with myelodysplasia

## REFERENCES


1 Bessis M General discussion I Is preleukemic states an adaequate designation? Blood Cells 2 347 1976
2 Blackburn E K Calender S T Dacie J V Doll R. Girdwood R H Mollin D L Saracci R Stafford J L Thompson R B Varadi S & Wetherley Mein G Possible association between pernicious anemia and leukemia. a prospective study of 1625 patients with a note on the very high incidence of stomach cancer Int J Cancer 2 163 1968
3 Block M Jacobson L O & Bethard W F Preleukemic acute human leukemia. JAMA 152 1018 1953
4 Deaton J G & Levin W C Systemic lupus erythematosus and actue myeloblastic leukemia Arch Intern Med 120 345 1967
5 Dreyfus B Preleukemic states Blood Cells 2 33 1976
6 Eichner E R. & Hillman R I The evolution of anemia in alcoholic patients Am J Med 50 218 1971

7 Finkel H E Brauer M J Taub R N & Dameshek W Immunologic aberrations in the Di Guglielmo syndrome Blood 28 634 1966
8 Frisch B & Lewis S M The bone marrow in aplastic anaemia Diagnostic and prognostic features J Clin Pathol 27 231 1974
9 Greenberg P L & Mara B The preleukemic syndrome Correlation of in vitro parameters of granulopoiesis with clinical features Am J Med 66 951 1979
10 Hast R Studies on human preleukaemia Clinical and Prognostic significance of sideroblasts in a regenerative anaemia with hypercellular bone marrow Scand J Haematol 21 396 1978
11 Hetzel P & Gee T S A new observation in the clinical spectrums of erythroleukemia. A report of 46 cases Am J Med 64 765 1978
12 Khaleeli M Keane W M & Lee G R Sideroblastic anemia in multiple myeloma A preleukemic change Blood 41 17 1973
13 Linman J W The preleukemic syndrome clinical and laboratory features natural course and management Blood Cells 2 11 1976
14 Linman J W & Bagby G C The preleukemic syndrome (hemopoietic dysplasia) Cancer 42 854 1978
15 Linman J W & Saarni M I The preleukemic syndrome Semin Hematol 11 93 1974
16 Mowat A G Connective tissue diseases In Clinical haematology 1 (ed M C G Israels and I W Delamore) p 573 Saunders London 1972
17 Papan A Papyannis A Kyrkou K et al Cytogenetic studies in preleukemia using the G banding technique Scand J Haematol 18 301 1977
18 Pierre R V Preleukemic states Semin Hematol 11 73 1974
19 Ross McIntyre O Multiple myeloma N Engl J Med 301 193 1979
20 Saarni M I & Linman J W Myelomonocytic leukemia disorderly proliferations of all marrow cells Cancer 27 1221 1971
21 — Preleukemia. The hematologic syndrome preceding acute leukemia. Am J Med 55 38 1973
22 Schaison G Najean Y Seligmann M Flandrin G Jacquillat C Weil M Cannat A & Ripault J Leucémie aigue à évolution prolongee et syndrome lupique Soc Fr d Hematol Seance du 18 novembre 1968 (1re partie)
23 Waldenstrom J G Benign monoclonal gammopathies In Multiple myeloma and related disorders (ed A Azar and M Potter) p 247 Harper & Row Hagerstown Maryland 1973

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# Acquired Pancytopenia in Relatives of Patients with Aplastic Anaemia

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**ABSTRACT** We studied relatives of adult patients with acquired aplastic anaemia Eight patients were found to have 11 family members with peripheral blood pancytopenia Six of the 11 affected relatives had diminished and four normal cellularity and one had hypercellularity of the bone marrow Thus, 19 persons in these eight families were affected In two families father and son were affected in four families brother and/or sister, in one family a brother and an aunt and in one a nephew of the index patient None of the patients or family members had congenital defects All patients were diagnosed at an adult age and furthermore also the mode of inheritance in some of the families seems to exclude Fanconi s syndrome It is concluded that relatives of patients with aplastic anaemia should be screened for manifestations of this syndrome of familial acquired blood pancytopenias

*Key words* aplastic anaemia familial pancytopenia

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Aplastic anaemia in childhood can be familial (4 5) or isolated Most cases seem to be constitutional but some may be acquired In adults however aplastic anaemia is usually considered to be isolated and acquired either drug induced postviral or idiopathic (15)

In a retrospective study of 55 adult patients with aplastic anaemia we noticed a familial occurrence of pancytopenia in eight This report describes these eight affected families

## PATIENTS AND METHODS

As many family members as possible of the eight patients with aplastic anaemia giving a positive family history of blood diseases were investigated

Routine peripheral blood counts were performed by standard laboratory methods Reticulocyte counts were corrected for haematocrit 48% in men and 41% in women Pancytopenia was defined as a haemoglobin level below 12 0 g/100 ml a number of polymorphonuclear neutrophils below 2 500/mm³ and of platelets below 120 000/mm³ at the same time

Bone marrow specimens were obtained by aspiration and by Yamshidi biopsy needle Aplastic anaemia was diagnosed when a patient with pancytopenia turned out to have a hypocellular or an acellular bone marrow

At physical examination special attention was paid to the known stigmata of Fanconi's syndrome short stature skin pigmentation microcephaly ocular anomalies cardiovascular anomalies and hypogonadism Skeletal malformations were investigated by physical examination and in most patients by X rays of the forearms and hands Information concerning the kidneys was obtained by i v pyelography

Additional investigations included Ham test and liver function tests In some patients cytogenetic studies were carried out with G banding

## RESULTS

Anaemia and leukopenia and/or thrombocytopenia were found in 11 out of 26 relatives of the eight index patients with aplastic anaemia Pertinent data on the index patients and their relatives with blood disorders are given in Table I

All index patients had pancytopenia and hypoplasia or aplasia of the bone marrow without evidence of malignancy extensive fibrosis or storage disease Pancytopenia was present in ten relatives bicytopenia in one Hypoplasia or aplasia of the marrow was found in six relatives normal cellularity in four and hypercellularity in one

Physical examination did not reveal Fanconi stigmata in any of the index patients or relatives In four patients no information concerning the kidneys could be obtained In all others i v pyelography was performed or the kidneys were found to be

Table I. *Data concerning index patients (A) and their relatives (B and C) with familial pancytopenia*

| Pat. no | Age (y) | Sex | Aetiology | Hb (g/100 ml) | Reticulocytes* (%) | Leukocytes (per mm³) | Neutrophils (%) | Platelets (per mm³) | Bone marrow |
|---|---|---|---|---|---|---|---|---|---|
| 1A | 30 | ♂ | Drug induced | 10.8 | 0.68 | 2 500 | 85 | 32 000 | Acellular |
| 1B | 27 | ♀ | Drug induced | 7.2 | 2.10 | 700 | 49 | 10 000 | Hypocellular |
| 1C | 19 | ♀ | Drug induced | 11.8 | 1.56 | 1 500 | 34 | 70 000 | Acellular |
| 2A | 81 | ♀ | Drug induced | 7.4 | 0.60 | 1 000 | 17 | 7 000 | Acellular |
| 2B | 67 | ♂ | Drug induced | 5.2 | 1.00 | 1 500 | 35 | 37 000 | Hypocellular |
| 3A | 36 | ♂ | Idiopathic | 9.7 | 1.32 | 3 000 | 50 | 25 000 | Hypocellular |
| 3B | 63 | ♂ | Idiopathic | 6.4 | – | 1 000 | 12 | 10 000 | Acellular |
| 4A | 42 | ♂ | Drug induced | 7.7 | 1.00 | 2 400 | 60 | 3 000 | Acellular |
| 4B | 29 | ♂ | Drug induced | 10.7 | 0.96 | 3 200 | 32 | 14 000 | Normocellular |
| 5A | 75 | ♂ | Idiopathic | 11.4 | 0.87 | 2 600 | 21 | 58 000 | Acellular |
| 5B | 71 | ♂ | Drug induced | 6.6 | 1.22 | 1 200 | 35 | 667 000 | Hypercellular |
| 5C | 72 | ♂ | Idiopathic | 5.7 | 2.08 | 4 000 | 56 | 42 000 | Normocellular |
| 6A | 27 | ♂ | Idiopathic | 4.0 | 0.71 | 700 | 31 | 3 000 | Hypocellular |
| 6B | 23 | ♂ | Idiopathic | 9.4 | 0.17 | 2 400 | 35 | 98 000 | Hypocellular |
| 7A | 17 | ♂ | Drug induced | 7.6 | 0.27 | 1 500 | 9 | 5 000 | Acellular |
| 7B | 37 | ♀ | Idiopathic | 7.4 | 0.56 | 1 500 | 52 | 10 000 | Normocellular |
| 7C | 17 | ♂ | Idiopathic | 7.7 | 0.33 | 650 | 22 | 3 500 | Acellular |
| 8A | 21 | ♂ | Idiopathic | 6.5 | 0.16 | 2 900 | 51 | 79 000 | Hypocellular |
| 8B | 50 | ♂ | Idiopathic | 10.8 | 1.25 | 3 500 | 53 | 110 000 | Normocellular |

* Corrected for haematocrit, 48% in men and 41% in women.

normal at autopsy. X rays of the forearms and hands were available in nine patients and were all normal. None of the patients had hepatomegaly, splenomegaly or multiple lymphadenopathy.

In the majority of patients, paroxysmal nocturnal haemoglobinuria was excluded by the history, by the Ham and sucrose haemolysis tests. None of the patients showed evidence of thalassemia or recent hepatitis.

Cytogenetic studies, performed in three patients, disclosed a Philadelphia chromosome during the terminal stage of the disease in one of them.

*Nine patients admitted use of potential aetiological agents.* They included tolbutamide, sulfadimethoxine, diphenylhydantoin, indomethacin, salicylates, hexachlorocyclohexane and other insecticides, nitrofurantoin, trimethoprim-sulfamethoxazole and Dolviran®. In one of these nine patients, 7A, we found serological evidence of a recent influenza A virus infection. No aetiological factor could be found in the others.

## CASE HISTORIES

### *Family 1*

*Patient 1A* was seen for the first time in 1967 at the age of 27 years because of diabetes mellitus. He was treated with diet and tolbutamide. Peripheral blood cell counts were normal until 30 years of age, when pancytopenia was discovered. Bone marrow biopsy revealed an acellular marrow. Tolbutamide treatment was discontinued. During the following 8 years the aplastic anaemia was unchanged. A recent bone marrow biopsy again showed an acellular marrow.

*Patient 1B*, a sister of 1A, had been treated repeatedly with sulfadimethoxine for urinary tract infections. In 1961 she exhibited pancytopenia. Her bone marrow was hypocellular. Therapy with vitamin $B_{12}$, folic acid and other vitamins, corticosteroids and anabolic steroids was unsuccessful. In the following years the patient experienced urinary and respiratory tract infections and allergic reactions to penicillin and iodopanoic acid. She died in 1967 of Escherichia coli septicaemia. A hypocellular bone marrow was found at autopsy.

*Patient 1C*, a sister of 1A, received phenobarbital, diphenylhydantoin and caffeine for epilepsy diagnosed at the age of 14 years. In 1964, at the age of 19, she developed spontaneous haemorrhages and was found to have a pancytopenia. Bone marrow biopsy revealed an acellular marrow. No treatment was instituted except discontinuation of diphenylhydantoin. The pancytopenia still persists.

Physical examination and peripheral blood cell counts in two brothers did not show any abnormalities.

### *Family 2*

*Patient 2A* was seen in 1975, at the age of 81 years, when she complained of fatigue. In the years prior to admission she had used indomethacin and salicylates for degenerative arthritis. Bone marrow biopsy disclosed an aplastic marrow. Prednisone therapy was unsuccessful. She [illegible] recently and the cause of death was not established by autopsy.

*Patient 2B*, a brother of 2A, was first admitted in 1965 at the age of 67 years. He suffered from degenerative

arthritis and used abundant analgesics especially acetyl salicylic acid Pancytopenia was diagnosed his bone marrow was hypoplastic He was treated with anabolic steroids and blood transfusions but the pancytopenia deteriorated gradually His bone marrow was repeatedly found to be hypoplastic The patient died in 1973

Results from physical examination and laboratory studies in four sisters and one brother were normal One sister and two brothers could not be examined

*Family 3*

*Patient 3A* was admitted for the first time in 1971 with a duodenal ulcer Thrombocytopenia (55 000/mm³) was found by chance A marrow aspiration was normal In 1972 at the age of 36 years he had developed pancytopenia and the bone marrow was hypocellular In 1977 a bone marrow biopsy showed an acellular marrow Results of cytogenetic studies of peripheral blood were normal

*Patient 3B* the father of patient 3A was seen at the age of 61 because of bleeding after a dental extraction He was anaemic without leukopenia or thrombocytopenia but two years later in 1967 pancytopenia was present Bone marrow biopsy showed an acellular marrow During hospitalization he received blood transfusions and corticosteroids but he died of myocardial infarction after a few weeks

Patient 3A has no siblings Information concerning brothers and sisters of patient 3B is lacking

*Family 4*

*Patient 4A* was admitted for the first time in 1973 at the age of 42 years because of pancytopenia and a bleeding diathesis As a farmer he had used the insecticide hexachlorocyclohexane in the months prior to hospitalization Bone marrow biopsy was acellular Treatment with anabolic steroids was unsuccessful the pancytopenia gradually deteriorated Six months later the patient died of Escherichia coli septicaemia and cerebral haemorrhage

*Patient 4B* a son of a sister of patient 4A was seen in our hospital in 1975 at the age of 29 years because of pancytopenia. Until this time he had been well As a farmer he also had frequently used insecticides Bone marrow biopsies until now show a normal cellularity The patient has not been treated and blood cell counts remain at the same level

Information concerning the other family members could not be obtained

*Family 5*

*Patient 5A* was first admitted in 1960 at the age of 62 years when the diagnosis of pernicious anaemia without leukopenia and thrombocytopenia was made Treatment with vitamin $B_{12}$ injections was started followed by a rapid response At 75 years of age pancytopenia was discovered The patient did not use drugs other than vitamin $B_{12}$ injections The bone marrow was acellular without megaloblastic haematopoiesis There was no response to treatment with vitamin $B_{12}$ or folic acid The pancytopenia deteriorated gradually and the patient died two years after the diagnosis of aplastic anaemia The cause of death was not determined by autopsy

*Patient 5B* a brother of patient A was seen in 1976 at the age of 71 because of fatigue and weakness He had been treated with nitrofurantoin and a combination of trimethoprim sulfamethoxazole for urinary tract infections The platelet count was normal but he was anaemic and had leukopenia A bone marrow aspiration and a biopsy showed a hypercellular marrow Cytogenetic bone marrow studies showed normal results Repeated bone marrow investigations in 1977 again showed hypercellularity in both erythropoiesis and myelopoiesis and also megakaryocytosis The anaemia and leukopenia persisted in the peripheral blood The patient died recently of pulmonary embolism

*Patient 5C* a brother of patient 5A was admitted to our hospital in 1968 at the age of 72 because of weakness and dyspnoea Pancytopenia was discovered Bone marrow aspiration and biopsy showed normal cellularity without megaloblastic haematopoiesis Treatment with vitamin $B_{12}$ folic acid and pyridoxin was not effective The blood pancytopenia deteriorated and the patient died in 1970 of gastric haemorrhage

Another brother has normal peripheral blood cell counts Two sisters died in 1918 at the age of 16 and 22 years of an influenza infection

*Family 6*

This is the only family with a history of consanguinity The parents of the index patient were first cousins

*Patient 6A* was seen in 1972 at the age of 25 years because of bleeding after tooth extraction He had leukopenia and thrombocytopenia but still a normal haemoglobin level The bone marrow biopsy showed normal cellularity In 1974 the pancytopenia deteriorated haemoglobin 4 0 g/100 ml WBC 700/mm³ platelets 3 000/mm³ The patient died in 1975 of Gram negative bacterial septicaemia At autopsy the bone marrow was found to be severely hypocellular

*Patient 6B* a brother of 6A was first admitted in 1971 at the age of 23 because of pallor and fatigue He had pancytopenia that deteriorated gradually The bone marrow was hypocellular Administration of vitamin $B_{12}$ folic acid pyridoxin and anabolic steroids was unsuccessful Three years later the bone marrow was again hypocellular In 1975 blast cells appeared in the peripheral blood The patient developed high fever and died Premortal cytogenetic studies of peripheral blood disclosed a Philadelphia chromosome in one of six analyzed metaphases Autopsy revealed a hypercellular bone marrow with predominantly large abnormal blast cells while pulmonary haemorrhages and fungal abscesses in the lungs and the brain were considered to be the cause of death

One brother had normal peripheral blood cell counts and a normal marrow on biopsy A sister had normal haemoglobin level and normal number of platelets but granulocytopenia 42% of 4 000 white cells/mm³ A bone marrow aspiration revealed normal cellularity

*Family 7*

*Patient 7A* had been well until 1977 when he was hospitalized for the first time at the age of 17 because of pancytopenia with fever and a bleeding diathesis In the week prior to admission he had used phenethicillin and

Dolviran* (salicylate phenacetin codeine caffeine and phenobarbital) for an influenza A virus infection Marrow aspiration and bone marrow biopsy showed a nearly acellular marrow Four weeks after admission the patient was transferred to the University Hospital Leiden for bone marrow transplantation He died of a disseminated fungal infection before bone marrow transplantation could be performed Autopsy revealed an acellular marrow

*Patient 7B* a paternal aunt of patient 7A was seen in 1976 at the age of 37 in the University Hospital Utrecht (K Punt) because of pancytopenia and hypermenorrhoea. The bone marrow was reported to show a normal erythropoiesis and myelopoiesis but no megakaryocytes Treatment with corticosteroids and anabolic steroids was unsuccessful

*Patient 7C* was investigated in 1977 during the hospitalization of his brother patient 7A At that time his peripheral blood cell counts were normal haemoglobin 12 8 g/100 ml leukocytes 4 000/mm³ with a normal differential count and platelets 203 000/mm³ In 1978 at the age of 17 he was admitted to the University Hospital of Leiden (J M Vossen) because of a bleeding diathesis and pancytopenia was diagnosed Bone marrow biopsy revealed an acellular marrow The patient died two months after bone marrow transplantation as a result of graft versus host disease

The father of patient 7A had normal haemoglobin level and platelet count WBC was 4 000/mm³ with 45% neutrophils

One sister of patient 7A who was HLA identical MLC negative also had a normal haemoglobin level and platelet count Her WBC was 5 600/mm³ with 33% neutrophils The mother and two sisters had normal peripheral blood cell counts Patient 7B has six sisters and five brothers (the father of patient 7A included) They have no clinical complaints Laboratory studies are however not available

*Family 8*

*Patient 8A* was seen in our hospital in 1970 because of pancytopenia. The bone marrow was hypocellular He was treated with anabolic steroids without any effect on haemoglobin level or the number of leukocytes Platelet count however was normalized

*Patient 8B* father of patient 8A was hospitalized because of pancytopenia in 1970 at the age of 50 He had a history of anaemia since 1955 Marrow aspiration and biopsy showed normal cellularity Two years later the patient died of a dissecting aneurysm The bone marrow was normal at autopsy

Patient 8A has one sister who has no complaints Information is lacking on family members of patient 8B

## DISCUSSION

Constitutional aplastic anaemia can be familial as described by Fanconi (5) in 1927 In all of these cases the blood disorder is associated with multiple congenital malformations Estren and Dameshek (4) described in 1947 a second group of children with familial aplasia but without congenital defects Chromosomal anomalies have been described subsequently in both groups (11 16)

Acquired aplastic anaemia has rarely been recorded in more than one member of a family Four pairs of twins with chloramphenicol induced aplastic anaemia have been reported (9, 10) Two siblings became pancytopenic after infectious hepatitis (2) In another family two sisters developed aplastic anaemia after administration of gold compounds (3) McLaren et al (8) reported two sisters who developed transient aplastic anaemia induced by methyprylon Three cases of idiopathic acquired aplasia in one family were described by Stolte (13) and one case of an affected mother and child is mentioned by Keiser (7) Though some of these patients were quite young it seems acceptable that the pancytopenia was acquired in all of them and not constitutional

Our patients also appear to have acquired and not constitutional disease The stigmata of Fanconi's syndrome were always absent In families 1 2 and 4 the history indicated exposure to a drug or chemical known to induce aplastic anaemia Other arguments for an acquired pancytopenia can be found in most other families the patients in families 3 and 5 presented initially without complete pancytopenia and members of families 6 and 7 passed the childhood without any sign of blood disease Furthermore one of the affected members of family 8 had not developed pancytopenia at 35 years of age We must therefore assume that all of our patients have acquired aplasia which is a rather rare disease throughout the world with an estimated incidence of about 4 8/1 000 000 persons/year (14) In our region the estimated frequency is approximately 4/1 000 000 persons/year implying that its occurrence in more than one family member and in so many families can hardly be coincidental It indicates that the members of the families described in this study probably have a genetically determined predisposition that makes them susceptible to development of bone marrow disease In nine of our patients the expression of this predisposition seems to be triggered by drugs No clear-cut toxic agents have been found in the others It might be suggested that these patients may react with bone marrow depression to agents not frequently associated with aplastic anaemia for instance salicylates and influenza infection

The expression of this predisposition to bone marrow disease in these families is most often in the

form of depression blood pancytopenia was present in all patients However, a hypo or aplasia of bone marrow elements was not found in all patients This may be due to a variability of marrow cellularity that is often found in aplastic anaemia (6) In patients 3A and 6A for example we initially found a normal bone marrow cellularity but later on a hypocellular and ultimately an acellular marrow It is therefore possible to observe decreasing cellularity when the disease deteriorates On the other hand the absence of hypocellularity in some relatives probably suggests a disorder different from the usual aplastic anaemia This is also suggested by the rather favourable prognosis of the patients and their relatives as far as median survival is concerned That the expression of the genetic predisposition may not always lead to depression of the bone marrow is suggested by the course of the disease in patients 6B and 5B In the former patient terminal evidence is found for the proliferation of blast cells in the latter patient proliferation of all cell lines of the bone marrow was present resulting in marked thrombocytosis This option of the bone marrow to react either with aplasia or proliferation is also known in benzene intoxication and in patients with the constitutional childhood aplasia of Fanconi who rather frequently develop some form of leukaemia (1)

The incidence of familial pancytopenia in our region seems to be remarkably high In the period during which the patients were seen 47 other patients without family history were admitted to our hospital This would mean that 15–20% of our patients with aplastic anaemia have family members with blood dyscrasias It is clear that this percentage is strikingly different from what is known in the literature Moreover in the future some of the isolated cases may probably turn out to be familial

The selection of our study population was of course biased by the fact that our attention was drawn to the families by at least one patient with clinical symptoms of pancytopenia and that only those families in which more than one patient was found are described in this report Nevertheless we are confronted with an unusual and striking familial occurrence of pancytopenia in adults Possibly a genetic defect could be the origin of these abnormalities Pancytopenia in children Fanconi s anaemia has a well defined genetic origin as an autosomal recessive gene mutation (12) Recessive heredity practically always implies a horizontal clustering of disease cases in families Among the eight families reported here there were four with an additional vertical expansion two families with father and son one family with an affected aunt of the index case and another with a nephew of the index case who had pancytopenia But when the methodical bias in the gathering of our material is taken into account there are too few patients to allow for a hypothesis of plain autosomal dominant inheritance

Considering that the age at onset varies widel and that a large proportion of our patients manifested the disease only after having been exposed to haematoxic agents it seems not unreasonable to consider the possibility of a dominant susceptibility mutant with variable penetrance or of a multifactorial inheritance with threshold The latter possibility is much more hypothetical than the former because any reliable data about population frequency or a suitable amount of family data are lacking up to now

After all it seems reasonable to suppose that if there is a genetic background to these cases of pancytopenia it is different from that of Fanconi s anaemia Consistent with this difference is the absence of the chromosomal fragility described in Fanconi s anaemia in the cases we could investigate

If in the future a genetic defect proves to be of any importance for the development of some cases of adult pancytopenia both the fact that the age distribution of our patients at the onset of the disease seems to be discontinuous and the marked similarity in the age at onset within the individual families would be interesting

For practical purposes it seems to be important to question patients with aplastic anaemia about their relatives and to examine these relatives for blood dyscrasias especially if they are considered as possible bone marrow transplantation donors

## REFERENCES


1 Bloomfield C D & Brunning R D Acute leukemia as a terminal event in nonleukemic hematopoietic disorders Sem Oncol 3 297 1976
2 Boga M & Szemere P A Infectious hepatitis and aplastic anaemia in two sisters Lancet 2 708 1971
3 Davidson L S P Davis L J & Innes J Studies in refractory anaemia Edinb Med J 50 355 1943
4 Estren S & Dameshek W Familial hypoplastic anemia of childhood Am J Dis Child 73 671 1947
5 Fanconi G Familiare infantile perniziosaartige

Table I *Incidence of OD and $W_{max}$ (mean ± S D ) before and after coronary bypass surgery related to degree of relief of chest pain*

| Postoperative chest pain | No of pats | OD (no of pats ) | | $W_m$ (W) | |
|---|---|---|---|---|---|
| | | Preop | Postop | Preop | Postop |
| Total relief | 6 | 2 | 3 | 68±30 | 97±24 |
| Improvement | 16 | 10 | 11 | 61±25 | 86±30 |
| Deterioration | 1 | 0 | 1 | 59 | 68 |

of 2 cm or more 3) Dysmotility (i e simultaneous contractions at a length of 10 cm at dry swallowing in 2 of 10 tested levels in the oesophagus) combined with a lower oesophageal sphincter hypotonia (i e a pressure gradient between the lower oesophageal sphincter and the oesophagus at the end phase of expiration of less than 9 mmHg) or combined with gastro-oesophageal reflux as diagnosed by a decrease in pH below 4 0 in the distal part of the oesophagus with application of an extra abdominal pressure of 100 mmHg 4) Severe dysmotility (i e simultaneous contractions at a length of 10 cm at dry swallowing in at least 3 of 10 tested levels in the oesophagus)

*A lower oesophageal sphincter hypotonia or gastro-*oesophageal reflux as single findings were not classified as OD Patients with preoperative OD were not systematically treated for this finding

The graded maximal exercise test was performed on a bicycle ergometer with continuous ECG registration during and 10 min after exercise Retrosternal chest pain or general fatigue were chosen as end points The maximal working capacity ($W_{max}$) was calculated (13)

Fisher s exact test and Students *t* test for paired observations were used for the statistical evaluation

## RESULTS

All patients had typical effort angina before operation At the follow up investigation chest pain had *disappeared in 6 patients improved in 16* and deteriorated in one patient

The capacity of daily life activity was estimated by interview and classified according to the functional criteria by the NYHA

Eighteen patients reported improvement of one or more degrees The single patient remaining in the most severe symptom group was the same man who had no benefit of the operation in other respects either (Fig 1)

The working capacity at bicycle ergometry was improved in 20 of the 23 patients (Table I) Thirteen of the 23 patients were disabled by chest pain also at the postoperative exercise test

Before bypass surgery OD was found in 52% of the patients and at the follow up in 65% The difference was not significant The results of the various oesophageal function tests are given in Table II With a few exceptions the same patients who had positive tests before had positive tests also after the operation The incidence of OD before and after operation was also unchanged in each group whether classified according to the degree of relief of chest pain (Table I) or to functional improvement according to the NYHA criteria (Fig 1) The incidence of oesophagus related symptoms other than chest pain was similar before and after operation (Table III)

## DISCUSSION

The frequency of OD is high in patients with chest pain of all origins including CHD (2 12 14 15) The considerable covariation between CHD and OD might hypothetically be explained by reflexes of oesophago-cardiac or cardio oesophageal type We have earlier studied the oesophago-cardiac reflex

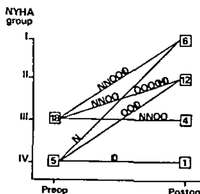



*Fig 1* Oesophageal findings before and after coronary bypass surgery in relation to NYHA functional
N = patient with normal oesophageal function O = oesophageal dysfunction pre and postoperatively ◐ = oesophageal dysfunction preoperatively ◑ = oesophageal dysfunction postoperatively

Table II *Findings at oesophageal manometry and at acid perfusion tests before and after coronary bypass surgery*

| * | Pos before and after | Neg before and after | Pos before neg after | Neg before pos after |
|---|---|---|---|---|
| Hernia | 6 | 16 | 0 | 1 |
| Reflux | 1 | 15 | 3 | 4 |
| Dysmotility | 10 | 10 | 1 | 2 |
| Acid perfusion test | 4 | 16 | 1 | 2 |

Table III *Oesophageal symptoms before and after coronary bypass surgery*

| | Before | After |
|---|---|---|
| Heart burn | 9 | 11 |
| Dysphagia | 8 | 7 |
| Surfeited after meals | 12 | 9 |
| Cough | 6 | 7 |

arch with afferent impulses from the oesophagus giving efferent impulses to the heart We infused acid into the oesophagus during continuous ECG registration but failed to induce ST T wave changes in this way (1)

In our patients with disabling angina pectoris the chest pain was most probably of cardiac origin If a cardio oesophageal reflex with afferents from the heart and efferents to the oesophagus were the ex planation of the high frequency of OD freedom from or relief of cardiac pain would reduce the frequency of OD In this study we made use of the observation that patients who have undergone coronary bypass graft operation are freed from chest pain or improve considerably whatever the true cause of this im provement may be (4 8) The frequency of OD and of oesophagus related symptoms other than chest pain was however unchanged compared to pre operative findings Thus no evidence was found supporting the hypothesis that the high frequency of OD in this patient group depends on a cardio-oeso phageal reflex

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## REFERENCES


1 Areskog M Tibbling L & Wranne B Oesophageal acid perfusion test as a complement to work test in patients with chest pain Acta Med Scand 201 559 1977

2 — Oesophageal dysfunction in non infarction coro nary care unit patients Acta Med Scand 205 279 1979

3 Del Regno F & Del Grosso V Stenocardia due to cholecystopathy Physiopathological and clinical find ings Minn Med 67 4203 1976

4 Gorlin R Coronary artery disease Major problems in internal medicine XI Saunders Philadelphia 1976

5 Morrison L M & Swalm W A Role of the gastro intestinal tract in production of cardiac symptoms experimental and clinical observations JAMA 114 217 1940

6 New York Heart Association Diseases of the heart and blood vessels 7th ed Little Brown & Co Boston 1973

7 Palmer E D The abnormal upper gastrointestinal vagovagal reflexes that affect the heart Am J Gastro enterol 66 513 1976

8 Preston T A Coronary artery surgery A critical review Raven Press New York 1977

9 Pyorala K Salmi H J Jussila J & Heikkila J Electrocardiographic changes during gastroscopy Endoscopy 5 186 1973

10 Rose G A The diagnosis of ischaemic heart pain and intermittent claudication in field surveys Bull WHO 27 645 1962

11 Serebro H A The prognostic significance of the viscerocardiac reflex phenomenon S Afr Med J 50 769 1976

12 Spandow O Sokjer H & Tibbling L Function of the lower oesophageal sphincter in a population selected at random A manometric radiological and questionnaire study Acta Otolaryngol 78 295 1974

13 Strandell T Circulatory studies in healthy old men Acta Med Scand (Suppl) 414 1964

14 Svensson O Stenport G Tibbling L & Wranne B Oesophageal function and coronary angiogram in patients with disabling chest pain Acta Med Scand 204 173 1978

15 Tibbling L & Wranne B Oesophageal dysfunction in male patients with angina like pain Acta Med Scand 200 391 1976

Table

Acta Med Scand 207 407–412 1980

# Patient Reaction to Information and Motivation Factors in Long-Term Treatment with Antihypertensive Drugs

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ABSTRACT In order to learn more about the patient–physician relationship, various aspects of information and communication, patient desires and complaints, a questionnaire form was mailed to three groups of male hypertensive patients Group A consisted of 264 patients response rate 61% (160 patients), originating from the employees' health service at two factories in Norway, and groups B (drug treated) and C (not drug treated) comprised 441 patients, response rate 82% (362 patients) and 328 patients, response rate 81% (265 patients) respectively, from the hypertension trial of the Oslo Study Information and/or communication failure was observed in all groups, more in group A than in groups B and C More information was wanted by 50–75% of the patients, especially in written form More than one half of the patients expressed complaints which might have been misinterpreted as being due to drug treatment With the exception of asthenia/drowsiness, impotence and podagra, which occurred more frequently in group B than in group C, the pattern of complaints was similar in these two groups

*Key words* hypertension treatment drug information communication motivation

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The outcome of drug treatment depends not only on a correct diagnosis and an appropriate prescription but also on the patient and his interpretation of the situation All these aspects are meant to be covered by the term drug utilization as defined by a WHO expert committee (22) Traditionally however too little attention has been paid to the reactions of the patients and to the crucial question whether they adhere to the medical instructions including the drug prescription Numerous studies (6 8 13 21) have revealed that patient non-compliance as regards drugs in many acute and chronic diseases is a dominant problem in medicine inside as well as outside institutions It has become increasingly evident that improved communication (14) especially between prescriber and patient is the most important single means of strengthening patient motivation for drug treatment (18)

Thus the present study which is part of a series of reports on drug utilization (1 2 3 4) deals with the patient–physician relationship various aspects of information and communication patient desires and complaints all related to hypertensive disease and to various types of long term preventive antihypertensive treatment

## PATIENTS AND METHODS

The present patient-oriented study comprising three male patient groups was based upon a questionnaire technique and took place during 1977

*Group A* consisted of 264 patients mean age 57 years (range 40–67) receiving drug treatment for hypertension Mean systolic and diastolic BPs at start of treatment were about 170 and 110 mmHg respectively The patients originated from the employees health services at Borregaard Industries Sarpsborg and at Norsk Hydro a.s Porsgrunn Norway For reasons of confidentiality comprehensive patient data could not be obtained from the health services

A questionnaire form (Appendix I) was mailed together with a covering letter and a return envelope to these 264 patients Answers were received from 160 patients (61%) No reminder was sent to this or the other two groups

*Groups B and C* comprised patients from the hypertension trial which is part of the Oslo Study an ongoing survey of cardiovascular diseases in males aged 40–49 years (mean 45) (17) The screening in the Oslo Study was performed in 1972–73 and the attendance was 65% in the age group 40–49 years (17) The hypertension trial is a controlled study to evaluate the drug treatment of border line and mild hypertension In 1972–73 the symptom free participants were randomly allocated to a drug treatment

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The article presents studies conducted in collaboration with Jon Efskind Borregaard Industries Sarpsborg and Helge Kjuus Norsk Hydro a s Porsgrunn Norway

Table I *Antihypertensive drugs administered to the patients in groups A and B*

| | No of patients | |
|---|---|---|
| | Group A ($n=160$) | Group B ($n=362$) |
| Diuretic only | 38 | 137 |
| β-Blocker only | 14 | 34 |
| α Methyldopa only | 7 | 8 |
| Prazosin only | 7 | |
| Total (%) | 41 | 49 |
| Diuretic + β blocker | 18 | 94 |
| Diuretic + hydralazine | 2 | 1 |
| Diuretic + α methyldopa | 24 | 75 |
| Diuretic + prazosin | 5 | |
| β-Blocker + hydralazine | 11 | 10 |
| β-Blocker + α methyldopa | 1 | 1 |
| β-Blocker + prazosin | 14 | 1 |
| Total (%) | 48 | 50 |
| Diuretic + β-blocker + hydralazine | 4 | |
| Diuretic + β-blocker + α methyldopa | 3 | |
| Diuretic + β-blocker + prazosin | 2 | 1 |
| Diuretic + α methyldopa + prazosin | 4 | |
| β Blocker + clonidine + prazosin | 1 | |
| Total (%) | 9 | |
| Diuretic + β-blocker + α methyldopa + hydralazine | 1 | |
| Diuretic + β blocker + prazosin + bethanidine | 1 | |
| Total (%) | 1 | |
| Unknown | 3 | |

group B ($n=406$) and an untreated control group C ($n=379$) Placebo was not used

The purpose of the trial was to evaluate the preventive effect of the drug including reduction of BP per se Accordingly no detailed information was given on other risk factors However brief information on overweight smoking and physical activity was given on request and then in the same way to the treated and untreated patients At each check up an appointment was made for the next consultation

The drug treatment consisted of hydrochlorothiazide (Dichlotride®) 50 mg/day Alpha methyldopa (Aldomet®) was added in increasing doses from 250 mg to 750 mg twice daily if the BP remained above 140/90 In the event of side-effects α methyldopa was replaced by propranolol (Inderal®) 40–160 mg twice a day When treated or observed for 4 years the patients received the same questionnaire form (Append x I) as patients in group A with some additional questi ns as illustrated in Appendix II The questionnaire form wa ly adju ted to fit the untreated control group C

During the four year su ey per od 4 pa ents in the untreated group C develope l marke l pe en on and

Table II *Information claimed to have been received from the physicians*

| Information about | Per cent of patients | | |
|---|---|---|---|
| | Group A ($n=160$) | Group B ($n=362$) | Group C ($n=$ 65) |
| Hypertension | 88 | 96 | 94 |
| Treatment | 62 | 93 | |
| Regular check ups | 65 | 92 | 88 |
| Diet | 38 | 74 | 71 |
| Smoking | 4[illegible] | 85 | 84 |
| Exercise | 33 | 76 | 76 |
| Stress | 29 | 65 | 62 |
| Drug(s) | 56 | 94 | |

antihypertensive treatment was started Six patients in group C died In this group the questionnaire was not mailed to 2 patients with advanced cancer and 2 with marked elevation of BP who were in the initial phase of their drug treatment Thus the questionnaire was mailed to 328 untreated persons in group C Six of the patients in the treated group B died Thus the questionnaire was mailed to 441 drug treated patients in group B The questionnaires were mailed from an institution formerly unknown to the patients The response rates in group B and C were 82 and 81% respectively

## RESULTS

### *Drug administration*

The antihypertensive drugs administered to the patients in groups A and B are listed in Table I In group A two thirds of the diuretics were thiazides mostly hydrochlorothiazides (Dichlotride® and Esidrex®) One third of the patients received chlorthalidone (Hygroton®) Hydrochlorothiazide (Dichlotride®) was used in group B Of the β-blockers oxprenolol (Trasicor®) and propranolol (Inderal®) were given to 35 and 43% respectively of the patients in group A while those in group B received propranolol Other drugs like psycholeptics analgesics and antihistamines were also given to 27% of the patients in group A to 14% in group B and to 9% in group C

### *Information received*

Tables II and III show what information regarding the disease and its treatment the patients claimed to have received from the physician Information about diet smoking exercise and stress (Table II) has been given to or perceived by less than half the patients in group A As regards drugs the communication seems even less appropriate in some

Table III *Drug information claimed to have been received from the physicians*

| Drug information about | Per cent of patients | |
|---|---|---|
| | Group A ($n=89$) | Group B ($n=314$) |
| Drug action | 49 | 76 |
| Side-effects | 33 | 66 |
| Regular use | 61 | 82 |
| Dose | 80 | 87 |
| When to take the drug | 78 | 91 |
| Additional use of other drug(s) | 11 | 32 |
| The drug(s) and alcohol | 16 | 45 |
| The drug(s) and car driving | 12 | 36 |

respects (Table III) About one half of the patients in all groups had picked up information on hyper tension from non medical sources (Table IV*a*) As to drug(s) however information sources other than the physician were uncommon (Table IV*a*) The most prominent information sources were newspapers radio and television followed by books pamphlets and weekly magazines (Table IV*b*) Only 1-2% of the patients claimed to have received any drug information at the pharmacies An appointment for the next consultation was perceived by 84% in group A by 97% in group B and by 91% in group C

*Information desired*

In groups A and C 72 and 63% respectively felt a need for more information In group B however more than half the patients seemed satisfied with the information received (Table V*a*) Of those who desired more information 70-90% were interested in learning more about the consequences of their elevated BP (Table V*b*) The wishes expressed for more drug information were mostly concerned with drug action and side-effects (Table V*b*) Of all those expressing a desire for more information about 80% wanted written information in addition to di rect verbal information About 20% of the patients also prefer drug information both from the physi cian and the pharmacist

*Complaints compliance special reactions*

The questionnaire to patients of the Oslo Study groups B and C included a question about a number of unspecific complaints or potential side effects/ adverse reactions (Appendix II) Such complaints were registered by 60% of the patients in the treated group B as compared to 53% of the non treated group C Concerning the individual com plaints in these two groups there was a clear distinction as far as headache/vertigo asthenia/drowsiness impotence and podagra were concerned (Fig 1) but no difference for such claims as GI disorders CNS disturbance skin irritation nasal stuffiness dryness of the mouth or cold extremities

Of the patients in this study 85% in group A and 90% in group B did not find it difficult to remember to take their medicine(s) Among those who re ported such a problem two thirds received more

Table IV *Information on hypertension and on the drug(s) obtained from other sources than the physi cian(s)*

| *a*) Information from other sources than the physicians(s) | Per cent of patients | | |
|---|---|---|---|
| | Group A ($n=160$) | Group B ($n=362$) | Group C ($n=265$) |
| On hypertension | 45 | 51 | 47 |
| On drug(s) | 21 | 12 | |

| *b*) Information sources | Per cent of patients | | | | |
|---|---|---|---|---|---|
| | Group A ($n=72$) | Group B ($n=166$) | Group C ($n=1$[illegible]$4$) | Group A ($n=34$) | Group B ($n=44$) |
| | On hypertension | | | On drug(s) | |
| Friends | 25 | 31 | 27 | 29 | 27 |
| Weekly magazines | 31 | 33 | 38 | 26 | 32 |
| Newspapers radio television | 85 | 84 | 82 | 71 | 55 |
| Books pamphlets | 50 | 55 | 42 | 32 | 55 |
| Pharmacy | | | | 3 | 18 |

*Table V. Information desired about hypertension and the drug(s)*

| | Per cent of patients | | |
|---|---|---|---|
| *a)* More information desired about | Group A (*n* = 160) | Group B (*n* = 162) | Group C (*n* = 65) |
| Hypertension | 71 | 44 | 63 |
| Drug(s) | 73 | 39 | |
| *b)* Type of information desired | Group A (*n* = 113) | Group B (*n* = 150) | Group C (*n* = 167) |
| How high is BP | 42 | 37 | 43 |
| The meaning of high BP | 73 | 75 | 66 |
| Does high BP mean reduced life activities | 83 | 77 | 70 |
| | (*n* = 117) | (*n* = 141) | |
| Drug action | 83 | 70 | |
| Dose | 37 | 20 | |
| When to take the drug(s) | 47 | 17 | |
| Side-effects | 85 | 79 | |

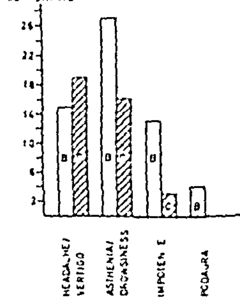



*Fig. 1.* Complaints and potential side-effects in patient groups B and C.

than one drug. The evening was said to be the time when medicine was most easily forgotten. The fact that the treatment of hypertension does require regular drug taking on a long term basis was apparently accepted by 95% of the patients in group A and 92% in group B. However, 3% in group A and 4% in group B preferred to conceal their situation from other people. In groups B and C, recruited from the Oslo Study, 80% stated that their participation in the study had meant an increased feeling of safety while 3 had become worried.

## DISCUSSION

### *Selection of patients*

In general, hypertension is as common among females as males. However, up to the age of 50 years the prevalence is higher in males than in females, the reverse being true at a higher age (2, 10). For practical reasons, the present study includes only men. Firstly, it became too complicated to collect a sufficient sample of relevant patients through various general practitioners (2). Secondly, most industries and other major labour activities in Norway are dominated by male employees or by relatively young females. Thus, it is quite clear that the present findings are not necessarily representative for the patients treated for hypertension in Norway, 75% of whom are cared for solely by general practitioners (2). Despite this, group A, which consisted of patients mostly handled by the employees' health service system and by general practitioners, probably does not deviate markedly from other hypertensive patients of corresponding age. In groups B and C, however, the patients were subject to an intensive health survey for 4 years and having established a close relationship to one of the internists attached to the scientific project, they must be considered somewhat atypical, thus serving as model groups. A response rate varying from 61% in group A to 82% in group B may also affect the representativity within each group. The 65% attendance at the screeing of the Oslo Study may further contribute to a selected group of hypertensive patients (groups B and C). Thus it is probable that groups B and C consist of patients having a better motivation for participation in a drug treatment/control programme than more unselected patients.

### *Questionnaires versus interview technique*

Postal questionnaires are a cheap and efficient method of collecting information. Compared with personal interview, the postal form is not influenced by an interviewer (23). However, simplicity in the formulation of questions and the length of the questionnaire are more crucial for postal than personal interviews (19). These factors may influence both

e response and the accuracy of the answers The amination of the questionnaires obtained in this dy indicates that the questions raised were well erstood

*eatment information and complaints*

he major features in the antihypertensive treatment in groups A and B are quite similar (Table I) he small differences detected may be explained by he different backgrounds of these groups Deficient information about diet smoking exercise and stress related to hypertension was apparent in roup A (Tables II and III) Such information is of mportance as smoking and a high level of serum holesterol seem to enhance the potential risk of gh BP in cardiovascular disease (16) An increase physical exercise and in the ability to relax may ave a favourable influence on the effect of antihypertensive drugs and stress may have the pposite effect (5 7) This study may indicate that he physicians do not pay sufficient attention to the elevance of these factors of hypertension

The finding that one half to two thirds of all atients in our study wanted more information corresponds to results of a Swedish study on antihypertensive treatment (11) The majority of those who desired more drug information wanted to know more about side-effects (Table V*b*) This is worth noticing as it may reflect personal experiences as well as a growing general scepticism towards drugs of all kinds However with the exception of asthenia/drowsiness impotence and podagra which occurred more frequently in the drug treated group B than in the non treated group C (Fig 1) (9) the pattern of complaints was similar in the two groups This underlines the difficulties in evaluating causal relationship between drug treatment and potential side-effects which may also reflect symptoms of the disease

The expressed wish for more information may reflect either insufficient information to or communication with the patients (14) Even if the information given was adequate the patients may not have understood or may have forgotten important parts of the instructions thus being left with inappropriate motivation However in the discussion of compliance/non-compliance factors such as motivation confidence between physician and patient and a meaningful appointment system are of central importance (12 15 20) In addition a simplification and adaptation of the drug regime to the patient s habits seems [illegible] groups B and C [illegible] between [illegible] more [illegible] the patients [illegible] should be checked [illegible] or supple[illegible] sary Key [illegible] tions should be given [illegible] a drug card in order [illegible] munication with the [illegible] macist In addition [illegible] phlets and/or books should be av[illegible]

## CONCLUSIONS

1 The study indicates that the information given at present to hypertensive patients by physicians and pharmacists is not sufficient A special need for more key note information in written form, both on the disease and its treatment seems to exist It is important that this written information is given in addition to not instead of, verbal information

2 The high frequency of apparent side effects as reflected by a number of complaints in the treated group was hardly due to the drug treatment as a similar pattern of complaints with some important exceptions also occurred in the non treated group

3 Accordingly more attention should be paid to these aspects among others in order to enhance patient confidence in motivation for and compliance with drug treatment and medical instructions related to hypertension

Appendix I *Summary of the questionnaire*

Mention the trademark of your antihypertensive drug(s) If you are using other drug(s) mention the trademark(s)

Has the physician informed you about

the blood pressure the meaning of treatment, the meaning of regular check-up diet smoking exercise, stress, drug action side-effects regular drug use, dosage, when to take the drug additional use of other drug(s), the drug(s) and alcohol the drug(s) and car driving?

If you have been informed about hypertension and/or antihypertensive drugs by others [illegible] which was the source of information

friends weekly magazines newspapers [illegible] books pamphlets pharmacist?

If you wish more information about [illegible] would it be about

blood pressure the meaning of hypertension [illegible] sion and life activities?

Do you want the information

written verbal both verbal and [illegible]

If you wish more information about your medicine would it be about
drug action dosage when to take the drug(s) side effect(s)?
From whom do you want this information
the physician the pharmacist both the physician and the pharmacist?
Do you find it difficult to remember to take your medicine?
When are drug(s) most easily forgotten
in the morning at noon in the evening at no special time?
Has the physician told you to come to a check up?
What does it mean to be on long term drug treatment
it is OK difficult to remember the drug(s) prefer to hide the drug use?

## Appendix II *Some questions added to the questionnaire sent to groups B and C*

Do you often suffer from
headache constipation diarrhoea nausea apathy depression insomnia drowsiness vertigo concentration difficulties impotence fatigue numbness dyspnoea cough skin irritation/rash nasal stuffiness dryness of mouth cold hands and feet podagra?
Has your participation in the Oslo study resulted in anxiety security neither anxiety nor security?

## ACKNOWLEDGEMENT

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## REFERENCES

1 Baksaas I Drug treatment with hypotensives with special emphasis on the relationship between prescribed and defined daily doses Medd Norsk Farm Selsk 40 69 1978
2 Baksaas I Fugelli P Halvorsen I K Lunde P K M & Næss K Prescription of hypotensives in general practice A study of 4 Norwegian counties in October 1975 Eur J Clin Pharmacol 14 309 1978
3 Baksaas Aasen I Lunde P K M Halse M Halvorsen I K Skobba T J & Stromnes B Drug dose statistics List of defined doses for drugs registered in Norway Norsk Medisinaldepot 1975
4 Baksaas Aasen I Stromnes B Halvorsen I K Halse M & Lunde P K M Legemiddelforbruksmonsteret i Norge Tidsskr Nor Laegeforen 97 170 1977
5 Benson H Rosner B A Marzetta B R & Klemchuk H M Decreased blood pressure in pharmacologically treated hypertensive patients who regularly elicited the relaxation response Lancet I 289 1974
6 Boyd J R Covington T R Stanaszek W F & Coussons R T Drug defaulting part II Analysis of noncompliance patterns Am J Hosp Pharm 31 485 1974
7 Boyer J L & Kasch F W Exercise therapy in hypertensive men JAMA 211 1668 1970
8 Caldwell J R Cobb S Dowling M D & De Jongh D The dropout problem in antihypertensive treatment A pilot study of social and emotional factors influencing a patient s ability to follow antihypertensive treatment J Chron Dis 22 579 1970
9 Dargie H J & Dollery C T Adverse reactions to diuretic drugs p 483 and Tester Dalderup C B M Hypotensive drugs p 461 In Meyler s side effects of drugs (ed M N G Dukes) Excerpta Med 8 1977
10 Eilertsen E & Humerfelt S The blood pressure in a representative population sample Acta Med Scand 183 293 1968
11 Eklund I H Wessling A & Åberg H Hypertonikers onskemål om läkemedelsinformation Nord Med 91 113 1976
12 Finnerty F A Jr Mattie E C & Finnerty F A Hypertension in the inner city I Analysis of clinic dropouts Circulation 47 73 1973
13 Fletcher S W Appel F A & Bourgeois M A Management of hypertension Effect of improving patient compliance for follow up care JAMA 233 242 1975
14 Harlem O K Medisinsk kommunikasjon In Medicinsk årbog p 227 Munksgaard Copenhagen 1978
15 Inui T S Yourtee E L & Williamson J W Improved outcomes in hypertension after physician tutorials A controlled trial Ann Intern Med 84 646 1976
16 Kannel W B McGee D & Gordon T A general cardiovascular risk profile The Framingham Study Am J Cardiol 38 46 1976
17 Leren P Askevold E M Foss O P Froili A Grymyr F Helgeland A Hjerman I Holme H Lund Larsen P G & Norum K R The Oslo Study Cardiovascular disease in middle aged and young Oslo men Acta Med Scand (Suppl) 588 6 1975
18 Mazzullo J M & Lasagna L Take thou But is your patient really taking what you prescribed? Drug Therapy 2 11 1972
19 Mork T Some problems related to the use of mail questionnaires J Chron Dis 23 399 1970
20 Sackett D L Haynes R B Gibson E S Taylor D W Roberts R S & Johnson A L Patient compliance with antihypertensive regimens Patient Counselling and Health Education 1 18 1978
21 Sigstad H Livindson A Hauge I J Ibsen T & Palm B Medikamentforbruk i hjemmene Relatert til dosering bivirkninger og komplikasjoner Tidsskr Nor Laegeforen 92 2319 1972
22 The selection of essential drugs WHO Tech Rep Ser 615 1977
23 Zeiner Henriksen T Comparison of personal interview and postal inquiry methods for assessing prevalence of angina and possible infarction J Chron Dis 25 433 1972

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# The Antihypertensive Effect of Prazosin on Mild to Moderate Hypertension, Changes in Plasma Volume, Extracellular Volume and Glomerular Filtration Rate

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**ABSTRACT** Changes in blood pressure, plasma volume (PV) ($^{1}$ I albumin space), extracellular volume (ECV) ($^{82}$Br space) and glomerular filtration rate (GFR) ($^{51}$Cr EDTA clearance) were measured in 12 patients with mild to moderate essential hypertension on placebo and during long term treatment with prazosin During the study, BP decreased from an average of 172/107 to 166/102 mmHg (n s ) PV increased from 3278 to 3324 ml (n s ) and ECV from 18360 to 18639 ml (n s ) GFR was almost unchanged 95 and 93 ml/min, prespectively An inverse significant correlation was found between the changes in mean BP and changes in ECV, i e fluid retention was demonstrated in patients with the smallest BP reduction It is concluded that inadequate BP response during treatment with prazosin may in part be due to fluid retention It is therefore suggested that prazosin should in principle be used together with a diuretic in order to prevent fluid retention

*Key words* prazosin body fluid compartments

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Prazosin is a postsynaptic α receptor blocking agent causing vasodilation but unlike other vasodilators the drug induces only minor reflex cardiac stimulation (4 11) The efficacy of prazosin as the sole agent in treatment of hypertension is not definitely established Evaluations in several controlled trials—compared wither with placebo or established agents—show differences in antihypertensive effects of the drugs It has been suggested that fluid retention might be a contributory factor in patients badly controlled on prazosin (9)

The present study was designed to measure the antihypertensive effect of prazosin alone and to evaluate the changes in body fluid compartments and glomerular filtration rate (GFR) during treatment

## PATIENTS AND METHODS

Twelve patients were admitted to the study eight men and four women with a mean age of 54 years (range 34–66) All had essential hypertension WHO stage I–II with supine systolic blood pressure (BP) of ≥165 and/or diastolic BP of ≥95 mmHg The investigational nature of the study was explained to the patients before obtaining their consent to participation

Three patients had a newly discovered hypertension while nine had been on antihypertensive treatment prior to the study On average they had been hypertensive for six years Prior to the investigations previously treated patients were withdrawn from any drug regimen for at least one month Then the patients were placed on placebo for a further month before the first measurement of plasma volume (PV) extracellular volume (ECV) and GFR was performed At the start of the study cardiac enlargement or left ventricular hypertrophy as detected by chest X ray and/or ECG were present in only one patient Eight patients had hypertensive retinal changes of grade I–II (Keith Wagener & Barker scale) All patients had normal renal function assessed by plasma creatinine determinations No abnormalities were found from urinalysis The patients were observed and investigated as out patients

BP was measured every other week during the first four weeks and at least as often thereafter with a mercury sphygmomanometer after 5–10 min rest in the supine position The diastolic pressures were read at the point at which the Korotkoff sounds disappear (phase V) The mean BP (MBP) was calculated as the diastolic BP plus one third of the pulse amplitude

Body fluids and renal function were estimated at 8 a m The patients had had nothing to eat or drink for at least 8 hours and they were kept in the supine position for at least half an hour before injection of the tracers and during the investigation The three isotopes were given i v in rapid succession as bolus injections Whole blood and plasma samples and urine samples for measurement of ECV were analyzed on the day of investigation while samples for determination of PV and GFR were stored at −20°C for 14 days allowing for disappearance of $^{82}Br^-$ and then analyzed simultaneously

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*Abbreviations* BP = blood pressure MBP = mean blood pressure GFR = glomerular filtration rate PV = plasma volume ECV = extracellular volume

*Table I Average changes in BP PV ECV and GFR during treatment with prazosin in the 12 patients studied*

Range given in parentheses

| | Control | Placebo | Prazosin | $p$ value | $p$ value* |
|---|---|---|---|---|---|
| Systolic BP (mmHg) | 176 (154–200) | 172 (143–190) | 166 (143–190) | n s | <0 01 |
| Diastolic BP (mmHg) | 108 (99–123) | 107 (95–119) | 102 (90–112) | n s | <0 05 |
| PV (ml) | | 3 278 (2 695–4 134) | 3 324 (2 673–4 524) | n s | |
| ECV (ml) | | 18 360 (15 637–25 143) | 18 639 (14 817–24 247) | n s | |
| GFR (ml/min) | | 95 (73–119) | 93 (73–116) | | |

* Control vs prazosin

PV was determined with [131]I labelled human albumin 6–8 μCi i v and blood samples were drawn 10 20 40 and 60 min later PV was calculated from the theoretical plasma radioactivity at time zero obtained by linear regression from the semilog radioactivity time graph and the gravimetrically determined amount of injected tracer (14) ECV was determined as the distribution volume of [82]Br as previously described (10) About 15 μCi of the tracer were injected and blood samples were taken after an equilibration period of 3½ hours

GFR was determined by [51]Cr EDTA using the single injection method described by Brochner Mortensen (5)

After the initial procedures the patients received prazosin as tablets in 3–4 daily doses increasing from 1 5 mg daily up to 20 mg daily The determinations of PV ECV and GFR was repeated after 2–6 months of treatment

Wilcoxon s test and Spearman rank correlation test were used for statistical analysis Values of $p<0\,05$ were considered statistically significant

## RESULTS

Compared to placebo prazosin alone caused only a minor and insignificant decrease in systolic as well as diastolic BP from 172 to 166 mmHg and from 107 to 102 mmHg respectively (Table I) MBP was reduced by more than 10% in only two patients The fall in BP was significant (systolic $p<0\,01$ diastolic $p<0\,05$) when values during control period before placebo treatment and during active treatment were compared Fig 1 presents the individual systolic and diastolic BPs measured during placebo and prazosin treatment

The mean prazosin dosage was 11.5 mg daily (range 3–20) For the group as a whole there was a small but insignificant increase in PV and ECV while GFR was almost unchanged (Table I Fig 2)

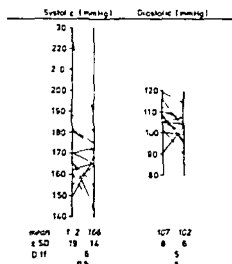



*Fig 1* Individual changes in BP during treatment with prazosin (placebo–prazosin)

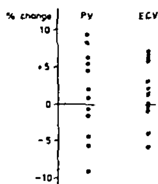



*Fig 2* Individual changes in PV and ECV during treatment with prazosin

A significant correlation was found between the change in MBP and the change in ECV (Fig 3*a*) but not between the change in MBP and the change in PV (Fig 3*b*) Thus almost no changes in fluid compartments were found in patients with the largest reduction of BP while an expansion of ECV was demonstrated in patients with unchanged BP

Side effects were mild (dizziness and weakness) and have been tolerated by the patients or have disappeared with continued treatment

## DISCUSSION

Fluid retention is a general problem in the treatment of hypertension with peripheral vasodilators (6, 7) as well as with other antihypertensive drugs In a study of 14 patients treated with prazosin alone Koshy et al (9) found that the antihypertensive properties of prazosin as the sole drug were more pronounced early after therapy than after 8 weeks of treatment PV did not change significantly in patients who responded well to prazosin while it increased significantly in patients with no or very slight decrease in BP Similarly Preston et al (15) *found no changes in blood volume in 10 patients* treated for one month with prazosin in an average dose of 6 9 mg/day In a study of 15 patients Ibsen et al (8) found after addition of prazosin to propranolol treatment a significant average increase in PV (8%) and ECV (5%) and a very modest decrease in BP of 11 mmHg systolic and 4 mmHg diastolic

It is reasonable to assume that the observation by Koshy et al of diminishing BP reduction during prazosin therapy may partly be due to fluid retention This assumption is supported by the present study where a small but significant correlation was found between changes in MBP and ECV However we found an insignificant increase in PV of *1 4% after 2–6 months of treatment* compared to a significant increase of 13 8% after 8 weeks of treatment observed by Koshy et al These discrepancies in PV could be due to the different observation periods Fluid retention during antihypertensive therapy may to some extent be a transient phenomenon and excess sodium may be excreted again during continued treatment Still some fluid retention may persist and thus play a role in the inadequate BP response (2) Racial differences in the studies could also be of importance (9 12)

GFR was not altered during prazosin therapy

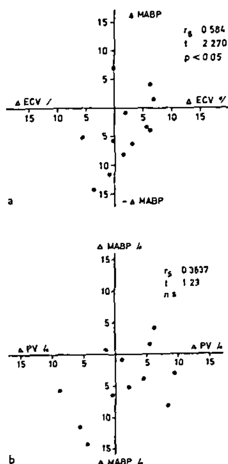



*Fig 3a* Changes in MBP and ECV in 12 patients with essential hypertension on prazosin alone

*Fig 3b* Changes in MBP and PV in 12 patients with *essential hypertension on prazosin alone*

Other investigators have similarly found no significant changes in creatinine clerance inulin clearance or p-aminohippurate clerance in hypertensive patients with normal renal function (9 15) or in ⁵¹Cr EDTA clearance in patients with renal impairment *(1) while others (8) found a decrease in* ⁵¹Cr EDTA clearance of 4% after addition of prazosin to propranolol treatment

Findings differ concerning the antihypertensive effect of prazosin as the sole agent Many investigators report some hypotensive effect of the drug (4) However only few other studies fulfil the criteria of a long run in period with placebo treatment (3 13 16)

In a double blind cross-over study between prazosin and hydrochlorothiazide Schirger and Sheps (16) found no BP reduction during prazosin

therapy when prazosin was administered as the sole agent Bolli et al (3) investigated prazosin versus placebo in a double blind cross-over trial in 12 hypertensive patients Nine patients were on combined therapy with two to four drugs only three were treated with prazosin alone They found a BP reduction of 17/8 mmHg in the lying posture during prazosin therapy compared to BP during placebo therapy Mroczek and Finnerty (13) studied the antihypertensive effect of prazosin compared to methyldopa and placebo in a randomized double blind trial on 60 patients with mild to moderate hypertension Twenty-one patients received prazosin alone in an average dose of 16 5 mg/day after an 8-week control period with placebo treatment A statistically significant reduction of 16/8 mmHg in supine BP was noted after 12 weeks of treatment

Our study and the studies of Koshy et al (9) and Ibsen et al (8) indicate that fluid retention may contribute to the inadequate BP control during treatment with prazosin We therefore suggest that in the treatment of hypertension prazosin should in principle be used together with a diuretic in order to prevent fluid retention

## REFERENCES


1 Bailey R R Prazosin in the treatment of patients with hypertension and renal function impairment Med J Aust (Special Suppl) 2 42 1977
2 Birkenhager W H & Schalekamp M A D H Control mechanisms in essential hypertension Elsevier Scientific Publishing Co Amsterdam Oxford and New York 1976
3 Bolli P Wood A J & Simpson F O Effects of prazosin in patients with hypertension Clin Pharmacol Ther 20 138 1976
4 Brogden R N Heel R C Speight T M & Avery G S Prazosin A reveiw of its pharmacological properties and therapeutic efficacy in hypertension Drugs 14 163 1977
5 Brochner Mortensen J A simple method for the determination of glomerular filtration rate Scand J Clin Lab Invest 30 271 1972
6 Finnerty F A Davidov M Mroczek W J & Gavrilovich L Influence of extracellular fluid volume on response to antihypertensive drugs Circ Res (Suppl) 1 1 1970
7 Gilmore E Weil J & Chidsey C Treatment of essential hypertension with a new vasodilator in combination with beta-adrenergic blockade N Engl J Med 282 521 1970
8 Ibsen H Rasmussen K Jensen H Æ & Leth A Changes in plasma volume and extracellular fluid volume after addition of prazosin to propranolol treatment in patients with hypertension Scand J Clin Lab Invest 38 425 1978
9 Koshy M C Mickey D Bourgoignie J & Blaufox M D Physiologic evaluation of a new antihypertensive agent. Prazosin HCL. Circulation 55 533 1977
10 Leth A & Binder C The distribution volume of $^{82}Br^-$ as a measurement of the extracellular fluid volume in normal persons Scand J Clin Lab Invest 25 291 1970
11 Lund Johansen P Hemodynamic changes at rest and during exercise in long term therapy of essential hypertension In Prazosin—Evaluation of a new antihypertensive agent Proceedings of a symposium held at the centre Interprofessionnel Geneva 1974 (ed D W K Cotton) Excerpta Medica 43 1974
12 Mitas J A Holle R Levy S B & Stone R A Racial analysis of volume–renin relationship in human hypertension Arch Intern Med 139 157 1979
13 Mroczek W J & Finnerty F A Prazosin a double blind evaluation In Prazosin—Evaluation of a new antihypertensive agent Proceeding of a symposium held at the centre Interprofessionnel Geneva 1974 (ed D W K Cotton) Excerpta Medica 92 1974
14 Parving H H & Gyntelberg F Transcapillary escape rate of albumin and plasma volume in essential hypertension Circ Res 32 643 1973
15 Preston R A O Connor D I & Stone R A Prazosin and renal hemodynamics Arteriolar vasodilation during therapy of essential hypertension in man J Cardiovasc Pharmacol 1 277 1979
16 Schirger A & Sheps S G Prazosin—New hypertensive agent JAMA 237 989 1977

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# The Natural History of Stroke in Diabetic Patients

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**ABSTRACT** A five year follow up of 53 diabetic patients admitted for their first stroke in 1972–73 has been performed They were compared with two groups of 53 non-diabetic patients each with cerebrovascular disease (CVD), one randomly selected and one matched with the diabetics for age, sex and diagnosis of CVD at discharge All patients could be traced at follow up The mean age at the time of first stroke was 66 5 years in male and 73 2 years in female diabetics Manifest diabetes was diagnosed in 19% during hospitalization for stroke, of the remainder, 74% had had diabetes since less than ten years In 85% of the diabetics there were no signs of severe angiopathy affecting eyes, kidneys or lower extremities The majority of diabetic as well as non diabetic CVD patients had a history of hypertension and/or heart disease Few were overweight Case fatality rate was significantly higher in diabetics than in non diabetics throughout the follow up ($p<0\ 01$ for diabetics vs matched non-diabetics, $p<0\ 001$ for diabetics vs randomly selected non diabetics) The presence of heart disorder predicted mortality in the diabetic subjects Surprisingly, hypertension diagnosed before stroke involved a more favourable long term prognosis in all three groups ($p<0\ 05$) The major causes of death in diabetic CVD patients were cardiac disorders (50%) and stroke (47%) Previous investigations have identified diabetes as a risk factor for stroke This study shows that diabetes also adversely affects the short term as well as the long term outcome in stroke

*Key words* cerebrovascular disease complications in diabetes diabetic macroangiopathy
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Diabetes mellitus confers excess mortality not only in microangiopathy but also in macrovascular disease Juvenile as well as maturity onset diabetes is associated with increased risks for ischaemic heart disease (32 43) and leg lesions (10 28) There is also a linkage between diabetes and cerebrovascular diseases (CVD) in that 1) diabetes involves enhanced morbidity and mortality in stroke (7 11 16 21 25 46) 2) among stroke patients most investigators (2 6 8 15 17 22 26 27 31 38 44) including ourselves (29) report a higher prevalence of diabetes than in the general population although low frequencies of manifest diabetes among CVD populations have also been observed (1 33) and 3) in autopsy series diabetes is found in a high proportion of patients with malacic brain lesions (37) and atherosclerosis of the cerebral vessels is reported to be more frequent in diabetic than in non-diabetic patients (4 19 37)

Despite the burgeoning mass of information on the course of diabetes including its vascular manifestations little is known about the natural history of CVD in diabetes Therefore the present study was undertaken to define some major characteristics of stroke as a complication in the diabetic patient

## PATIENTS AND METHODS

This study was made in retrospect and included three groups of patients admitted to the Department of Medicine Umeå University Hospital for their first attack of stroke including transient ischaemic attacks (TIAs) during 1972 and 1973 *Group A* All 53 patients with manifest diabetes and stroke admitted during this period *Group B* 53 non-diabetics with stroke matched with the diabetics for sex age and diagnosis of CVD at discharge according to the International Classification of Diseases (ICD) *Group C* 53 randomly selected non-diabetic patients with stroke Comparisons between groups A and B serve to isolate the influence of diabetes after the first stroke On the other hand some characteristics peculiar to diabetics with stroke as delineated from a general CVD population are exposed in comparison between groups A and C

CVD was diagnosed on clinical grounds The diagnosis was accepted only if focal neurological symptoms or signs had been documented or if findings at angiography cere

*Abbreviations* CVD = cerebrovascular disease ICD = International Classification of Diseases TIAs = transient ischaemic attacks BP = blood pressure

Table I *Sex and age distribution in diabetic and non diabetic patients with stroke (n=53 in each group)*

| | Sex (%) | | Age (y mean±S E M) | | |
|---|---|---|---|---|---|
| | *Males* | *Females* | *Males* | *Females* | *Total* |
| Diabetics | 26 | 74 | 66 5±3 3 | 73 2±1 5 | 71 5±1 4 |
| Matched non-diabetics | 26 | 74 | 66 8±3 2 | 73 2±1 4 | 71 5±1 1 |
| Non matched non-diabetics | 34 | 66 | 72 6±2 0 | 72 4±1 3 | 72 5±1 3 |

brospinal fluid examination or autopsy were confirmatory Recent advances in diagnostic procedures such as computer assisted tomography and spectrophotometry of the cerebrospinal fluid were not available at the time of the index events In view of the inaccuracy of differential diagnosis between different types of stroke based on bed side data (3) no attempts were made to delineate haemorrhagic from ischaemic brain lesions

The medical records were extracted and the following items considered 1) hypertension known before the stroke 2) cardiac disorders as follows documented myocardial infarction angina pectoris atrial fibrillation history or presence of congestive heart failure medication with heart glucosides and cardiac enlargement as judged by chest roentgenogram 3) other serious disorders in the past or at present 4) severe overweight—since data on height were not always available severe overweight was arbitrarily defined as more than 90 kg in men and more than 60 kg in women 5) major complications during the hospital stay 6) discharge to home or to further institutional care 7) cause of death

As regards the diabetics further data obtained from the medical records included 1) duration of diabetes 2) late diabetic complications defined as severe retinopathy (verified by an ophthalmologist) causing total or partial loss of vision nephropathy with constant proteinuria leg lesions with gangrene ulcer or amputation Reliable data on diabetic neuropathy were not available

Information on fatal and major non fatal medical events during the following five year period were obtained from hospital records and general physicians death certificates and telephone interviews with surviving patients All patients surviving the first stroke could be traced at follow up In no patient had specific secondary preventive measures (pharmacological or surgical) been instituted

The items recorded at follow up were 1) possible onset of diabetes in groups B and C 2) recurrent manifest strokes 3) length of survival 4) causes of death

Student s $t$ test and the $\chi^2$ test were used as statistical standard procedures Since the study was completed after a five year follow up data on those surviving at that time were considered censored Therefore for statistical evaluation of survival data Gehan s non parametric test for comparing survival distribution with censored data (18) was used To facilitate calculations survival data were grouped at intervals as follows (months) 0–3 4–12 13–60 61–

## RESULTS

### *Sex and age distribution*

There was a female preponderance among diabetic as well as non-diabetic patients (Table I) the female male ratio being close to 4 1 in the diabetics The diabetic patients were somewhat younger than the randomly selected non-diabetics The difference could be attributed to the fact that diabetic males were affected by stroke at a significantly younger age than non-diabetic males ($p<0\ 001$) while diabetic females were slightly older than randomly selected non-diabetic females with stroke Diabetic males with stroke were also significantly younger than diabetic females ($p<0\ 001$)

### *Duration and complications of diabetes*

The distribution of duration of diabetes at the time of the first stroke is shown in Fig 1 In ten patients (19%) manifest diabetes was diagnosed during hospitalization for stroke The majority of the others (74%) had had the disease for less than ten years Only two of the patients (4%) had onset of insulin-dependent diabetes before the age of 40

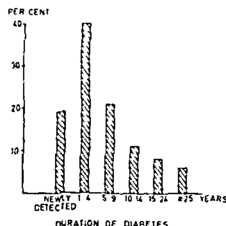



*Fig 1* Duration of known diabetes in 53 patients admitted for stroke

Diabetics afflicted by CVD had generally a low incidence of severe retinopathy or nephropathy

Table II *Late diabetic complications in 53 diabetics with stroke*

| | N | % |
|---|---|---|
| Severe retinopathy | 3 | 6 |
| Nephropathy | 5 | 9 |
| Leg lesions | 5 | 9 |
| None of the above | 45 | 85 |

(Table II) Both patients with insulin-dependent juvenile onset diabetes had however azotaemia as well as advanced retinopathy with reduced visual acuity In 85% of the diabetics there were no signs of severe retinopathy nephropathy or gangrenous lower leg lesions Ischaemic heart disease will be considered separately below

*Concomitant disorders*

Hypertension had frequently been diagnosed before the stroke in diabetics as well as non-diabetics (Table III) A meaningful comparison of the frequency of elevated blood pressure (BP) in the diabetics and their matched controls was not possible since the ICD classification for CVD also includes the presence or absence of hypertension When compared with the randomly selected non-diabetics with stroke it appeared that the diabetics had a higher frequency of hypertension (74 vs 57%) Antihypertensive medications had been prescribed to all but one of the patients with known elevated BP A reasonable assessment of patient compliance and actual control of BP was however not possible to perform

A large proportion of the patients in all three groups had overt heart disease (Table III) Again the diabetics showed the highest prevalence Atrial fibrillation was present in a substantial proportion of patients in all three groups (21–28%) indicating embolization from the heart as a possible cause of stroke

None of the diabetics with stroke whose weights were recorded was grossly overweight (Table III) A low prevalence of overweight was observed also among non-diabetic CVD patients

When previously diagnosed disorders were extracted a notable finding was that a history of thyroid disorder (goiter hyperthyroidism or primary hypothyroidism) was recorded in more than 10% of the total stroke population with no significant differences between diabetic and non-diabetic patients Of the diabetics 15% had a history of renal or urinary tract disorder 11% gallbladder disease and 8% achlorhydria all percentages well above those observed in the two non-diabetic populations with stroke (data not shown)

*Events during initial admission for stroke*

In association with the acute CVD event a substantial proportion of all patients had complications involving the heart (myocardial infarction or congestive heart disease arrhythmias not included) or the urinary tract (infection or incontinence) Similar frequencies of cardiac (21–23%) and urinary tract (19–23%) complications were recorded in diabetics and in their matched controls

The case fatality rate during the initial hospitalization was 25% in the diabetics 19% in their matched non-diabetic controls and 15% in the randomly selected non-diabetic patients with stroke

Reliable information on the extent of residual disability could not be obtained from the medical records Whether the patient returned to home or was discharged to further institutional care could however be expected to mirror the integrated medical functional and social status of the patient Among the randomly selected non-diabetics 57% of surviving subjects were discharged to home This figure was significantly higher ($p<0.05$) than in diabetics (35%) and matched non-diabetics (33%)

*Long term survival*

Fig 2 shows that the cumulative survival was lower in diabetics with stroke than in the two non-diabetic CVD populations throughout the five year observation period (diabetics vs matched non-diabetics $p<0.01$ diabetics vs randomly selected non-dia

Table III *Prevalence (%) of cardiovascular disorders and overweight in diabetic and non diabetic patients with stroke (n=53 in each group)*

| | Diabetics | Matched non diabetics | Non matched non diabetics |
|---|---|---|---|
| Previously diagnosed hypertension | 74 | 55 | 57 |
| Heart disease | 81 | 72 | 66 |
| Atrial fibrillation | 21 | 28 | 28 |
| Overweight* | 0 | 13 | 11 |

* Data on weight were not available in all patients and are based on recordings in 38–47 of 53 patients in each group

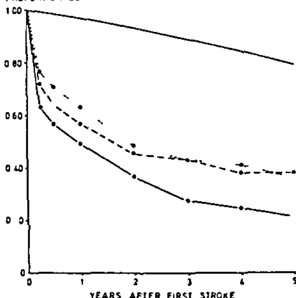



*Fig. 2.* Five year survival after first stroke in diabetics (●—●) matched non-diabetics (● ●) and randomly selected non-diabetics (● ●) $n=53$ in each group. —=Cumulative survival in a cohort of the general population matched with the diabetics for age and sex (data from Swedish Statistical Year Books).

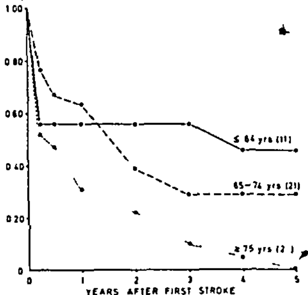



*Fig. 3.* Five year survival in 53 diabetics classified according to their age at the time of stroke. Number of patients in each age group is given in parentheses.

betics $p<0.001$). Half of the diabetic patients died within one year after the index event, and only one of five was alive after five years. There was no significant difference in survival between the two non-diabetic groups.

Among possible predictor items for survival we analyzed 1) age, 2) history of hypertension, and 3) history of heart disorder.

The well recognized association of reduced long term survival with increasing age (39) was present in diabetic subjects with CVD as well (Fig. 3). The survival during the first three months after stroke was, however, poor also in diabetic patients below the age of 65.

As shown in Fig. 4, hypertension diagnosed before the onset of first stroke did not seem to affect adversely the survival after stroke. On the contrary, more patients were alive in all three groups after five years if hypertension had been diagnosed before the first stroke ($p<0.05$ in each of the three groups).

The presence of cardiac disease, on the other hand, resulted in an unfavourable prognosis in CVD patients with as well as without diabetes (Table IV).

### *Recurrent stroke and causes of death*

Among diabetics discharged alive after their first stroke, 32% had at least one more cerebrovascular accident (TIAs not included) during the follow up period. The corresponding figures were 29% for the matched non-diabetics and 40% for the randomly selected CVD patients.

Table V shows the causes of death. The final diagnoses were based on autopsy reports in a similar proportion (62–79%) of the cases in the three patient groups. There were no decisive differences in the profile of diagnoses between diabetics and non-diabetics. Heart disorders and strokes were the dominating causes of death.

### *Mortality in relation to hypertension*

Since, contrary to our expectations, the presence of hypertension involved a more favourible prognosis after stroke in both diabetics and non-diabetics (see above), this observation was analyzed more in detail. It appeared that the excess mortality among patients without hypertension was confined to death from CVD, while death from cardiac disease was as frequent in hypertensives as in non hypertensives (Table VI). When the total stroke population was considered, the proportion of subjects with

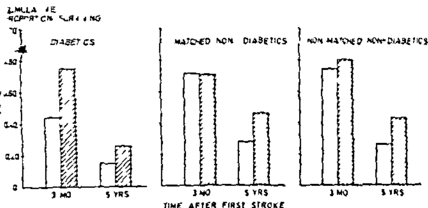



Fig. 4 Survival three months and five years after first stroke in relation to absence (□) or presence (▨) of known hypertension before stroke. n=53 in each group

known cardiac disease did not differ significantly among hypertensives and non hypertensives (65 vs 69%). Atrial fibrillation was present in a slightly smaller proportion of patients with (23%) than without (30%) hypertension. Hypertensive patients had a somewhat lower mean age than the normotensives: 1.5 years lower in diabetics, 4.2 years lower in matched non diabetics and 1.3 years lower in randomly selected non diabetics.

*Development of diabetes in the controls*

In only one of the non diabetic patients with stroke did manifest diabetes mellitus develop later during the follow up period (three years after the first stroke).

## DISCUSSION

It is held by some investigators that hospital medical records are too incomplete to permit meaningful evaluation of medical data and care (14, 35). Before recent advances in diagnostic procedures (including computer assisted tomography and spectrophotometry of the cerebrospinal fluid), hospital discharge diagnoses of CVD did not adequately reflect pathological diagnoses (3) or even data available in the medical records (5). Nevertheless some investigators have experienced that medical records from university and community hospitals contain enough information to define many essential characteristics of CVD populations, such as underlying diseases, neurological signs and laboratory data (5). With these considerations in mind, we have included only easily recognizable and identifiable data that were recorded on all or most patients.

Diabetes has been described as a disease of premature aging (47). This precocious aging would en

Table IV *Short and long term survival in relation to absence (A) or presence (P) of heart disease in diabetic and non diabetic patients with stroke (n=53 in each group)*

| | Survival (%) | | | | |
|---|---|---|---|---|---|
| | 3 months | | 60 months | | |
| | A | P | A | P | p value* |
| Dia[illegible] | 90 | 56 | 40 | 16 | <0.05 |
| Ma[illegible]betics | 80 | 68 | 40 | 37 | >0.05 |
| No[illegible] diab[illegible]ics | 83 | 74 | 56 | 26 | <0.05 |

* Calculated for absence vs presence of heart disease by the use of Gehan's non parametric test as described in Methods

Table V *Causes of death in diabetics and non diabetics with a first stroke and during a five year follow up (n=53 in each group)*

*n* denotes no. of deaths during follow up. More than one cause of death was recorded in several patients

| | Deaths (%) | | |
|---|---|---|---|
| | Diabetics (n=42) | Matched non diabetics (n=33) | Non matched non diabetics (n=34) |
| First stroke | 33 | 42 | 30 |
| Recurrent stroke | 14 | 21 | 30 |
| Heart disorders | 0 | 24 | 42 |
| Pulmonary embolism | 7 | 21 | 9 |
| Bronchopneumonia | 17 | 21 | 12 |
| Uraemia | 2 | 0 | 0 |
| Others | 2 | 6 | 12 |

Table VI *Relation between presence (P) or absence (A) of previous hypertension and death from CVD and cardiac disease among patients with stroke during a five year follow up*

| | No of deaths during follow up | Death from CVD (%) | | Death from cardiac disease (%) | |
|---|---|---|---|---|---|
| | | P | A | P | A |
| Diabetics | 42 | 31 | 57 | 38 | 43 |
| Matched non diabetics | 33 | 29 | 52 | 14 | 16 |
| Non matched non-diabetics | 34 | 23 | 57 | 33 | 17 |

compass aspects of the immune system the connective tissue the blood vessels etc (41) Of particular interest in the context of CVD are measurements of transit time in the retinal circulation which has been found to be related to chronological age In persons with overt or chemical diabetes there is a precocious aging of this function (45) *This may indicate that the cerebral arteries are similarly* affected by diabetes The precociously aged cerebral vessels would perhaps make diabetics more prone to develop a stroke than non diabetics

The diabetic stroke patients of the present study had predominantly maturity onset diabetes of fairly short duration In a substantial number of patients diabetes was diagnosed during admission for stroke It could not be decided whether these subjects had previously had an undiagnosed diabetes or the CVD-related trauma precipitated manifest diabetes in predisposed individuals It is known that *stroke involves profound stress reactions with* enhanced catecholamine mobilization (13) and hyperglycaemia in the early phase also in patients without manifest diabetes (34) and without impaired glucose tolerance (29)

*The present findings indicate that diabetes affects* adversely the outcome after a stroke When short term outcome is considered the mortality was higher during initial admission among the diabetics than non-diabetics Furthermore the majority of the surviving diabetics were discharged to institutional care The reason for this aggravated course of CVD in diabetes is not immediately evident Experimental studies in monkeys (36) and rats (42) have however demonstrated that hyperglycaemia increases the extent of ischaemic brain lesions during anoxic conditions Whether a similar situation pertains in the diabetic patient remains to be clarified

An alternative explanation of the excess early mortality in the diabetics could be an increased frequency of haemorrhages and oedema formation due to small vessel lesions relative to brain infarctions when compared with non-diabetics Since diabetic angiopathy includes bleeding from retinal vessels and exudate formation other vessels also originating from the internal carotid arteries could conceivably be affected in an analogous manner In experimental as well as human diabetes increased oedema formation has been demonstrated in the vicinity of necrotic lesions (20 30)

The long term prognosis in our stroke patients appears to be predominantly determined by the progressive course of macrovascular disease leading to death from heart disease or recurrent stroke This applies to both diabetic and non-diabetic patients Cardiac disease was of crucial prognostic importance in our stroke patients It was present already before stroke in most subjects it was associated *with an increased* early mortality after stroke and it was the major cause of death during the five year follow up A close relation between CVD and ischaemic heart disease has previously been amply demonstrated in non-diabetic subjects (2 8 24 33 40)

The prognostic implications of hypertension are less evident Hypertension is uniformly singled out as the most important risk factor for stroke (23) BP was also a predictor of short term prognosis in a relatively young population of non-diabetic males affected by stroke (39) In the present investigation hypertension was diagnosed before stroke in most of the patients a particular high proportion (74%) being recorded in the diabetics It is not evident whether this observation is due to an enhanced prevalence of hypertension in diabetics or just reflects *a more frequent* and *thorough* medical check up in diabetics Whether or not hypertension is more frequent among diabetic than non-diabetic individuals is still a matter of controversy (9)

To estimate the presence of hypertension we chose to *record elevated BP diagnosed by a physi*cian before the stroke BP is often elevated immediately after a stroke and then gradually returns to lower values (12) *Therefore high BPs recorded on* admission do not necessarily reflect manifest hypertension

We noted that in diabetics as well as in non

diabetics known hypertension was associated with an improved rather than an aggravated long term prognosis after the first stroke No unifying explanation of this observation could be found The excess mortality among non hypertensive individuals was confined to death from CVD This raises the question whether after a first stroke hypotensive episodes have a role to play in further deleterious manifestations of CVD Cerebral vessels severely compromised by degenerative lesions may need a high systemic BP to permit adequate perfusion of the brain

## ACKNOWLEDGEMENTS

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## REFERENCES


1 Acheson J Factors affecting the natural history of focal cerebral vascular disease Q J Med 40 25 1971
2 Aho K Incidence profile and early prognosis of stroke Epidemiological and clinical study of the 286 persons with onset of stroke in 1972 and 1973 in a South Finnish urban area. M D thesis Helsinki 1975
3 Alderson M R & Meade T W Accuracy of diagnosis on death certificates compared with that in hospital records Br J Prev Soc Med 21 22 1967
4 Alex M Baron E K Goldenberg S & Blumenthal H T An autopsy study of cerebrovascular accident in diabetes mellitus Circulation 25 663 1962
5 Carpenter R R Rodgers K D & Reed D E Diagnostic information available in university and community hospital medical records Patients with cerebrovascular disease Stroke 3 739 1972
6 Conant R G Perkins J A & Ainley A B Stroke morbidity mortality and rehabilitative potential J Chron Dis 18 397 1965
7 Deckert T Poulsen J E & Larsen M Prognosis of diabetics with diabetes onset before the age of thirtyone I Survival cause of death and complications Diabetologia 14 359 1978
8 Dyken M L Precipitating factors prognosis and demography of cerebrovascular disease in an Indiana community a review of all patients hospitalized from 1963 to 1965 with neurological examination of survivors Stroke 1 261 1970
9 Editorial Diabetes and hypertension Lancet 2 138 1978
10 Ellenberg M Diabetic foot NY State J Med 73 2778 1973
11 Entmacher P S Root H F & Marks H H Longevity of diabetic patients in recent years Diabetes 13 373 1964
12 de Faire U Britton M Helmers C & Wester P-O Blood pressure during the acute phases of cerebrovascular disease Acta Med Scand (Suppl) 621 27 1978
13 Feibel J H Hardy P M Campbell R E Goldstein M N & Joynt R J Prognostic value of stress response following stroke JAMA 238 1374 1977
14 Fessel W J & van Brunt E E Assessing quality of care from the medical record N Engl J Med 286 134 1972
15 Frithz G Studies on cerebrovascular stroke I Epidemiology of first time strokes in persons under 70 years of age Ups J Med Sci 80 141 1975
16 Garcia M J McNamara P M Gordon T & Kannel W B Morbidity and mortality in diabetics in the Framingham population Sixteen year follow up study Diabetes 23 105 1974
17 Gertler M M Rusk H A Whiter H H Leetma H E & Ehrenkrantz, M Ischemic cerebrovascular disease The assessment of risk factors Geriatrics 23 135 1965
18 Gross A J & Clark V A Survival distributions Reliability applications in the biomedical science pp 243–250 Wiley New York 1975
19 Grunnet M L Cerebrovascular disease diabetes and cerebral atherosclerosis Neurology 13 486 1963
20 Hallmans G Lithner F & Hagg E Early cutaneous reactions to local traumatization with heat in alloxan diabetic rats Ups J Med Sci 83 17 1978
21 Hammond E C & Garfinkel L Coronary heart disease stroke and aortic aneurysm Factors in the etiology Arch Environ Health 19 167 1969
22 Harmsen P & Tibblin G A stroke register in Goteborg Sweden Acta Med Scand 191 463 1972
23 Kannel W B Current status of the epidemiology of brain infarction associated with occlusive arterial disease Stroke 2 295 1971
24 Kannel W B Dawber T R Cohen M E & McNamara P M Vascular disease of the brain—epidemiological aspects The Framingham study Am J Public Health 55 1355 1965
25 Kessler I I Mortality experience of diabetic patients A twenty-six year follow-up study Am J Med 51 715 1971
26 Kuller L Anderson H Petersen D Cassel J Spiers P Curry H Paegel B Saslaw M Sisk C Wilber J Millward D Winkelstein W Jr Lilienfield A & Seltser R Nationwide cerebrovascular disease morbidity study Stroke 1 86 1970
27 Larsson T Mortality from cerebrovascular disease In Stroke Skandia International Symposia (ed A Engel & T Larsson) pp 15–40 Nordiska Bokhandelns Forlag Stockholm 1967
28 Lithner F Lesions of the legs in diabetics and in patients with familial amyloidosis and polyneuropathy Acta Med Scand (Suppl) 589 1976
29 Lithner F Asplund K Hagg E Strand T & Wester P-O Forekomst av diabetes mellitus hos patienter med cerebrovaskulär sjukdom (Occurrence of diabetes mellitus in patients with cerebrovascular disease) Acta Soc Med Suec 87 200 1978
30 Lithner F Hallmans G & Hietala S-O Cutaneous haemorrhages and gangrenes localized to the low

er limbs in patients with collagen diseases and in diabetics Ups J Med Sci 83 145 1978
31 Louis S Stroke prone individual NY State J Med 66 2772 1965
32 Luft R Efendić S & Cerasi E Prediabetes diabetes and atherosclerosis Some considerations In Early phases of coronary heart disase Skandia International Symposia pp 223-232 Nordiska Bokhandelns Forlag Stockholm 1973
33 Marquardsen J The natural history of acute cerebrovascular disease A retrospective study of 769 patients Acta Neurol Scand (Suppl) 38 1969
34 Melamed E Reactive hyperglycemia in patients with acute cerebrovascular disease J Neurol Sci 29 267 1976
35 Murnaghan J H & White K L Hospital patient statistics problems and prospects N Engl J Med 284 822 1971
36 Myers R E Anoxic brain pathology and blood glucose Neurology 26 345 1976
37 Najenson T Mendelson L Schbansky H Don R & Sandbank U Diabetes and cerebrovascular accidents Isr J Med Sci 6 598 1970
38 Petlund C F Prevalence and invalidity from stroke in Aust Agder county of Norway M D thesis Universitetsforlaget Oslo 1970
39 Rabkin S W Mathewson F A L & Tate R B The relation of blood pressure to stroke prognosis Ann Intern Med 83 15 1978
40 Robinson R W Demirel M & LeBeau R J Natural history of cerebral thrombosis nine to nineteen year follow up J Chronic Dis 21 221 1968
41 Rosenbloom A L Nature and nurture in the expression of diabetes mellitus and its vascular manifestations Am J Dis Child 131 1154 1977
42 Siemkowicz E & Hansen A J Clinical restitution following cerebral ischemia in hypo- normo and hyperglycaemic rats Acta Neurol Scand 58 1 1978
43 Sievers J Blomqvist G & Biörck G Studies on myocardial infarction in Malmö 1935 to 1954 VI Some clinical data with particular reference to diabetes menopause and heart rupture Acta Med Scand 169 95 1961
44 Sjöström Å Hospitalized cases of stroke in a Swedish hospital region In Stroke Skandia International Symposia (ed A Engel & T Larsson) pp 41-50 Nordiska Bokhandelns Förlag Stockholm 1967
45 Soeldner J S Christacopoulos P D & Gleason R E Mean retinal circulation time as determined by fluorescein angiography in normal prediabetic and chemical-diabetic subjects Diabetes 25 903 1976
46 Stallones R A Dyken M L Fang H C H Heyman A Seltzer R & Stamler J Epidemiology for stroke facilities planning Stroke 3 360 1972
47 Whittingham S Mathews J D Mackay I R Stocks A L Ungar B & Martin F I R Diabetes autoimmunity and aging Lancet 1 763 1971

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# Evaluation of Tests for Gilbert's Syndrome

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**ABSTRACT** The effect of caloric restriction (400 kcal for 24 hours) on serum total and unconjugated bilirubin was studied in 30 subjects with Gilbert's syndrome and in 22 patients with different liver diseases The method could not completely differentiate between Gilbert's syndrome and liver disease, but an increase in unconjugated bilirubin of 15 μmol/l or more supports the former diagnosis This limit gave a 100% specificity and a sensitivity for males of about 90% and for females of about 40% I v nicotinic acid caused similar rises of unconjugated bilirubin as reduced caloric intake in eight subjects with Gilbert's syndrome, but most of the subjects preferred the latter test Results from erythrocyte porphyrin determination in seven subjects with Gilbert s syndrome gave some support to the presence of dyserythropoiesis

*Key words* Gilbert s syndrome hyperbilirubinemia fasting nicotinic acid erythrocyte porphyrins

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Gilbert s syndrome is probably the most common cause of isolated hyperbilirubinemia in Sweden and certainly so in outpatients The syndrome which has also been called Gilbert s disease constitutional hepatic dysfunction idiopathic unconjugated hyperbilirubinemia and icterus juvenilis intermittens is characterized by a high concentration of serum unconjugated bilirubin in the presence of normal serum concentrations of conjugated bilirubin and normal results from routine tests for liver damage The presence of hemolysis is excluded by the finding of a normal reticulocyte count In the asymptomatic young outpatient these findings are usually considered sufficient for the diagnosis However since isolated unconjugated hyperbilirubinemia has been described in a wide range of common diseases e g heart diseases with or without decompensation biliary diseases pancreatitis alcoholism cirrhosis malignancy and after hepatitis (8) there may sometimes be a need of confirmatory diagnostic procedures Needle biopsy of the liver or kinetic studies with radioactive bilirubin (2) are impractical for the investigation of such a common benign condition which may in effect be regarded only as a normal variant (1)

Two relatively simple tests for diagnosis of Gilbert s syndrome have been described A reduction of the caloric intake may lead to pronounced increase in the serum concentration of unconjugated bilirubin in subjects with Gilbert s syndrome whereas the increase in normal individuals usually is only slight (5) Owens and Sherlock (13) evaluated this test in ten subjects with Gilbert s syndrome who had hyperbilirubinemia at the time of the study The authors concluded that an increase of 100% or more usually seen within 24 hours after reducing the daily caloric intake to 400 kcal suggests that unconjugated hyperbilirubinemia is due to Gilbert s syndrome In 12 patients with liver disease there was a mean increase of 8% in unconjugated bilirubin the largest increase found being 50% Since two other subjects with Gilbert s syndrome who had normal basal plasma bilirubin concentration showed a response similar to that in the normal subjects the authors concluded that the test cannot be used to diagnose Gilbert s syndrome at a time when the plasma bilirubin concentration is normal

The other test the nicotinic acid test (6) is based upon Mattei s observation (10) that i v nicotinic acid causes a rise in serum unconjugated bilirubin concentration

Davidsson et al (4) compared the two tests An increase of 100% in unconjugated bilirubin as a result of reduced caloric intake occurred in only four of 13 subjects 12 of whom had basal hyperbilirubinemia With the nicotinic acid test 12 of 16 individuals with Gilbert s syndrome showed a more pronounced rise than the most pronounced rise in

the controls This would indicate that the latter test has a higher sensitivity albeit still unsatisfactorily low Patients with liver disease were not investigated in this study

We have performed the reduced caloric intake test in 30 subjects with Gilbert s syndrome and compared the results with those from patients with liver disease and no or moderate hyperbilirubinemia In eight of the subjects with Gilbert s syndrome we also performed the nicotinic acid test as done by Davidsson et al (4) In seven of these subjects we also measured erythrocyte porphyrins before and after the test The reason for this analysis was the recent observation (11) of a slow incorporation of radioactive iron into circulating erythrocytes in subjects with Gilbert s syndrome suggested by these authors to indicate that ineffective erythropoesis might be a contributory factor for the hyperbilirubinemia We also analyzed serum ferritin C reactive protein and orosomucoid as aids in the evaluation of the erythrocyte porphyrin data

## PATIENTS AND METHODS

The 30 subjects (seven females) with the diagnosis of Gilbert s syndrome were all healthy at the time of the study The hyperbilirubinemia had been detected by chance at health control or in connection with symptoms that were later explained by the presence of a non-hepatic disorder The subjects had normal values of serum conjugated bilirubin alkaline phosphatases and aminotransferases and normal blood reticulocyte counts Their initial total bilirubin concentrations ranged between 11 and 66 μmol/l (mean 30 6 normal 3 21) seven subjects having normal basal concentrations

The nicotinic acid test was carried out in eight of these 30 subjects (one female) with basal bilirubin concentrations of 16–51 μmol/l (mean 33 3) One of these subjects had normal basal values

The reduced caloric intake test was also performed in 22 patients with liver disease (10 with alcoholic cirrhosis 4 with non alcoholic 3 with alcoholic fatty liver 1 with hemochromatosis 1 with primary biliary cirrhosis 2 with primary and 1 with secondary liver cancer) who had basal bilirubin concentrations of 5 4–81 μmol/l (mean 34 1) Eight patients had normal basal values

All subjects with Gilbert s syndrome were outpatients whereas all with liver disease were hospitalized during the study Samples for basal bilirubin assay were drawn after an overnight fast For the reduced caloric intake test the subjects were prescribed a 400-kcal diet for 24 hours The nicotinic acid test was also carried out after an overnight fast A slow i v injection of 50 mg of nicotinic acid in 1 ml of saline was given over 30 sec Blood samples for the assay of bilirubin porphyrins ferritin C reactive protein and orosomucoid were drawn before and 180 min after the injection

Reference values for erythrocyte porphyrin concentrations in normal individuals were obtained from the analysis of samples from 51 healthy students 18 females (mean age 27 years range 21–38) and 33 males (mean age 26 years range 21–44)

Total and conjugated serum bilirubin concentration were measured according to Nosslin (12) with minor modifications Erythrocyte porphyrins (mainly protoporphyrin) were measured fluorometrically after extraction with acetic acid-ethylacetate followed by addition of dilute aqueous hydrochloric acid (14) Serum ferritin was assayed by an immunoradiometric method (Ramco Laboratories Houston Texas USA) The precision and accuracy obtained with this method in our hands is described briefly elsewhere (9) Serum orosomucoid and C reactive protein were analyzed by electroimmunoassay (7) For statistical comparison we used Wilcoxon s test

## RESULTS

*Reduced caloric intake test* In the 30 subjects with Gilbert s syndrome the mean (±S D ) serum unconjugated bilirubin concentration rose from 25 0±12 0 to 54±24 0 μmol/l The mean rise (29 0±20 4) was highly significant ($p<0\ 001$) (Fig 1) The mean rise in the seven subjects who had normal basal concentrations of total bilirubin (21 7±8 0) was also highly significant and did not differ significantly from that in the 23 subjects with initially increased bilirubin levels (31 2±22 6) Increases of more than 100% in unconjugated bilirubin concentrations were noted in six of the seven subjects with normal basal values for total bilirubin concentration and ten of the 23 with hyperbilirubinemia The rise was more pronounced ($p<0\ 001$) in males (33 2±21 5 μmol/l) than in females (15 1±4 4 μmol/l)

No significant change in unconjugated serum bilirubin concentration was found in the 22 patients with liver disease In three patients with liver disease the increases (10 1 11 and 11 μmol/l) exceeded the slightest increase (9 2 μmol/l) in the subjects with Gilbert s syndrome

*Nicotinic acid test* The mean increase in unconjugated bilirubin after administration of nicotinic acid (27 5±18 3 μmol/l) did not differ from the increase after the caloric restriction test observed in the same subjects (39 0±23 4) In only one subject was the total bilirubin normal before nicotinic acid and the rise in this subject was 8 2 μmol/l Only one subject showed a rise of more than 100% in unconjugated bilirubin

The erythrocyte porphyrin concentrations were normal in five of the seven subjects studied and slightly elevated in two (Fig 2) The latter two

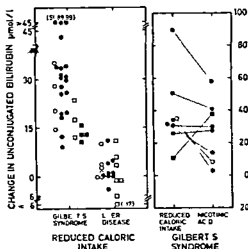



*Fig 1* Changes in serum unconjugated bilirubin concentration induced by reduced caloric intake and i v nicotinic acid in subjects with Gilbert s syndrome and in patients with liver disease Filled symbols indicate individuals with basal hyperbilirubinemia O ●= males □ ■= females Note the difference in scales between the two parts of the figure

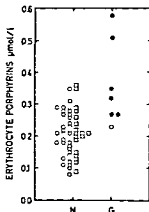



*Fig 2* Erythrocyte porphyrin concentrations in normal subjects (N) and subjects with Gilbert s syndrome (*G*) Symbols as in Fig 1

subjects did not differ from the other five in their response to nicotinic acid or caloric restriction None of them showed signs of acute inflammatory reaction clinically or from the assays of serum orosomucoid and C reactive protein or had any evidence of iron deficiency as judged from blood hemoglobin serum iron iron binding capacity and serum ferritin concentrations Bone marrow examination was not carried out Serum ferritin was 66 1±9 7 µg/l (range 50–77) before and 71 9±15 3 µg/l (range 49–90) after nicotinic acid administration All values were normal

## DISCUSSION

Normal controls were not studied in the present investigation for two reasons Firstly subjects with suspected Gilbert s syndrome consult a doctor because they have an abnormal finding viz hyperbilirubinemia It seems therefore rather needless to look for a more complicated test that can distinguish between subjects with normal and with abnormal serum bilirubin The doctor s dilemma is to find out whether the subject has a liver disease or not a question that is often raised not only because of unexplained symptoms but also because of fear of exposure to hepatotoxic chemicals The second reason which is coherent with the first is that subjects with Gilbert s syndrome should probably also be considered normal admittedly at the extreme of the normal distribution The data presented by Bailey et al (1) strongly suggest that they belong to the same population as those with constantly normal serum bilirubin levels

We agree with the statement by Davidsson et al (4) that increases in unconjugated bilirubin of 100% or more after reduced caloric intake are far from common in Gilbert s syndrome For practical reasons we studied the subjects only after 24 hours We felt that a more prolonged caloric restriction may be difficult to accomplish in outpatients On the other hand we did not observe more pronounced rises of unconjugated bilirubin after nicotinic acid as a matter of fact five of the eight tested subjects showed more pronounced rises after reduced caloric intake Since in addition four subjects felt markedly uncomfortable after the nicotinic acid (one had headache for 48 hours following the injection) and five out of the eight subjects definitively preferred the reduced caloric test we feel that the latter test should be the first choice when there is a need to verify the diagnosis of Gilbert s syndrome

We then come to the question which change after reduced caloric intake is required to dismiss a suspicion of liver disease Judged from our data the increase in unconjugated bilirubin after a 24-hour 400-kcal diet should be well above 10 µmol/l to support a dismissal of liver disease A decision limit of 15 µmol/l might be used in the present study it

gives a specificity of 100% It should be pointed out though that the number of individuals studied with e g liver cirrhosis was small The sensitivity for males with this limit was about 90% (20/23) but only 40% (3/7) for females This finding of a sex difference is of interest It may be noted that according to Bailey et al (1) in a normal population high bilirubin levels are far more common in males than in females

In addition to the diagnostic value of the test its psychological value in anxious patients should not be disregarded As regards the performance of the test we suggest that the two samples should be assayed simultaneously to eliminate the effect of interassay variation Thus the basal sample should be stored in e g a dark tube until the second sample is available

Five of the seven individuals with Gilbert s syndrome had erythrocyte porphyrin values within the upper normal limit whereas two had values above this limit These data lend some support to the proposal by Metreau et al (11) that dyserythropoiesis is present in subjects with Gilbert s syndrome More adequate iron kinetic studies (3) than those performed so far in subjects with Gilbert s syndrome are however required before this question can be settled

## REFERENCES


1 Bailey A Robinson D & Dawson A M Does Gilbert s disease exist? Lancet 1 931 1977
2 Berk P D Bloomer J R Howe R B & Berlin N I Constitutional hepatic dysfunction (Gilbert s syndrome) A new definition based on kinetic studies with unconjugated radiobilirubin Am J Med 49 2? 1970
3 Cavill I Ricketts C & Jacobs A Radioir n a erythropoiesis methods interpretation and c?? application Clin Haematol 6 583 1977
4 Davidson A R Rojas-Bueno A Thompson R H & Williams R Reduced caloric intake a nicotinic acid provocation tests in the diagnosis Gilbert s syndrome Br Med J 2 480 1975
5 Felsher B F Rickard D & Redeker A G T reciprocal relation between caloric intake and the c gree of hyperbilirubinemia in Gilbert s syndrome Engl J Med 283 170 1970
6 Fromke V L & Miller D Constitutional hepa dysfunction (CHD Gilbert s disease) a review w special reference to a characteristic increase and p. longation of the hyperbilirubinemic response nicotinic acid Medicine 51 451 1972
7 Laurell C B Quantitative estimation of protein electrophoresis in agarose gel containing antibo Anal Biochem 15 45 1966
8 Levine R A & Klatskin G Unconjugated hy bilirubinemia in the absence of overt hemolysis An Med 36 541 1964
9 Lindstedt G Lundberg P A Bjorn Rasmussen & Magnusson B Serum ferritin assay and the dia nosis of iron deficiency anaemia in hospital patient Lancet 1 205 1980
10 Mattei C Sui vari aspetti della curva bilirubinemi da carico di acido nicotinico nei normali e ne epatopazienti Minerva Med 37 308 1946
11 Metreau J M Yvart J Dhumeaux D & Bert elot P Role of bilirubin overproduction in revealu Gilbert s syndrome is dyserythropoiesis an importa factor? Gut 19 838 1978
12 Nosslin B The direct diazo reaction of bile pigmen in serum Experimental and clinical studies Scand Clin Lab Invest (Suppl) 49 1 1960
13 Owens D & Sherlock S Diagnosis of Gilbert syndrome Role of reduced caloric intake test Br Me J 3 559 1973
14 Piomelli S A micromethod for free erythrocyte po phyrins J Lab Clin Med 81 932 1973

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# Correlation between Prognostic Factors and Blood Variables in Osteosarcoma

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**ABSTRACT** Prognostic factors and the blood chemistry were analyzed prior to treatment in 33 consecutive patients with primary osteosarcoma The only variables of blood chemistry with values outside the normal range were alkaline phosphatase and ESR, which were increased There was a correlation between certain prognostic factors and between some of these and a number of the blood variables The degree of malignancy tended to be lower in the female than in the male patients Distally located tumors tended to be smaller and were assessed with a less marked effect on the blood variables than those located proximally

*Key words* osteosarcoma prognostic factors blood chemistry interferon

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During the last few years the efficacy of a variety of adjuvant forms of treatment of osteosarcoma has been examined (5) In view of the absence of randomized patient populations an assessment of the comparability of series as regards certain prognostic factors has been proposed In previous studies we have examined the importance of various prognostic factors in a consecutive series of osteosarcoma patients receiving interferon adjuvant to conventional treatment of the primary tumor (4) The blood chemistry was examined initially and at regular follow ups (1) The results of the initial blood tests were analyzed for a possible relationship with various prognostic factors

## PATIENT SERIES

The study population was a consecutive series of 33 patients with primary osteosarcoma and no demonstrable metastases who received treatment at the Karolinska Hospital from 1972 to 1979 The treatment of the primary tumor including interferon adjuvance has been reported elsewhere (1 14) None of the patients had a previous history of serious illness and in none had the tumor disease affected the nutritional state Weight and height lay within the reference ranges (3)

*Prognostic factors* The factors considered in the analysis are listed in Table I Ten of the patients were females The mean age for the series was 20 years and only one patient was over 30 (a woman aged 62) The mean duration of the symptoms prior to biopsy was 4 months The mean initial tumor size represented by the largest diameter on a radiograph was 10 cm All the tumors were located in the long bones or the pelvis in 13 patients distal to the elbow or the knee The open biopsy specimens were assessed by one and the same pathologist together with another independent pathologist The tumors were classified in accordance with the schedule of Broders et al (2) and Dahlin (8) There was a predominance of osteoblastic high-grade tumors

*Blood chemistry* All blood tests were performed at the Department of Clinical Chemistry Karolinska Hospital All samples were collected at the initial examination i e prior to interferon therapy and surgical manipulation of the tumor The tests performed are listed in Table II

## METHODS

Relationships between the prognostic factors and between these and the blood variables were examined by means of computerized correlation analysis Pearson s correlation coefficient $r$ and $p$ values were calculated

## RESULTS

The reference values for the blood variables are those accepted as normal at the Karolinska Hospital These are given as 95% confidence interval (Table II) Except for alkaline phosphatase activity and ESR there was no significant difference be

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Table I *Prognostic factors*

| | No of pats | Mean | S D | Range |
|---|---|---|---|---|
| Males | 23 | | | |
| Females | 10 | | | |
| Age (y ) | | 20 | 9 | 8–62 |
| Duration of symptoms prior to presentation (mo ) | | 4 | [illegible] | 4–20 |
| Size of tumor (cm) | | 10 | 3 5 | 4–[illegible] |
| Type of tumor | | | | |
| Osteoblastic | 21 | | | |
| Chondroblastic | 4 | | | |
| Fibroblastic | 8 | | | |
| Grade of tumor | | | | |
| IV | 17 | | | |
| III | 10 | | | |
| II | 6 | | | |
| I | 0 | | | |
| Location of tumor | | | | |
| Proximal | 20 | | | |
| Distal | 13 | | | |

tween the recorded values and the reference values (*Table II*) The sex distribution age duration of the symptoms tumor size and location and histologic picture were typical of primary osteosarcoma (7 10) A statistically significant correlation was found for the following variables

*Sex* The female patients exhibited a longer duration of symptoms prior to the first consultation ($r=0\ 36$ $p<0\ 05$) a higher level of IgG ($r=0\ 49$ $p<0\ 01$) a lower Hb concentration ($r=0\ 35$ $p<0\ 05$) and a higher incidence of low grade tumors ($r=0.32$ $p<0\ 05$) than the males Six of the 10 women and 2 of the 23 men had a fibroblastic tumor

*Age* Older patients had a longer duration of the symptoms ($r=0\ 77$ $p<0\ 01$) and higher levels of alanine aminotransferase (ALAT) ($r=0\ 65$ $p<0\ 01$) and IgA ($r=0\ 88$ $p<0\ 01$) than the younger

*Duration of symptoms* was negatively correlated to the malignancy grade of the tumor ($r=0\ 35$ $p<0\ 05$) and positively correlated to the S ALAT level ($r=0\ 63$ $p<0\ 001$) and IgG ($r=0\ 36$ $p<0\ 05$)

*Tumor size and location* Distally located tumors were significantly smaller ($r=0\ 48$ $p<0\ 01$) and accompanied by higher serum Hb levels ($r=0\ 41$ $p<0\ 01$) and a slower ESR ($r=0\ 43$ $p<0\ 01$) than the proximally located ones

*Pathological findings* A lower grade of malignancy ($r=0\ 50$ $p<0\ 01$) and a lower level of haptoglobin ($r=0\ 34$ $p<0\ 05$) were found in the fibroblastic than the osteoblastic and chondroblastic tumors

*Serum alkaline phosphatases* There was no correlation between alkaline phosphatase activity and the other prognostic variables examined

## DISCUSSION

In our 33 patients with primary osteosarcoma the prognostic factors recorded initially before any treatment had been given are typical of this disease (7) No case showed evidence of tumor spread on admission and all the patients were in good general condition That the grade of malignancy was lower in the tumors of female than male patients is indicated by the predominance of low grade tumors and the long lasting history of symptoms The high level of IgG observed in the female patients is consistent with the findings in a previous study in which low grade tumors were found to be associated with high levels of gamma globulin (13) The Hb concentration would be expected to be lower in females than

Table II *Blood variables recorded at initial examination*

| | Mean ± S D | Range | Reference values |
|---|---|---|---|
| ESR (mm/h) | 16±17 | 1–60 | 1–15 |
| Leukocyte count (10⁹/l) | 6 9±2 1 | 3 7–11 4 | 4–9 |
| Hb (g/l) | 142±13 | 121–180 | 120–170 |
| Blood platelets (10⁹/l) | 322±112 | 145–615 | 150–400 |
| Albumin (g/l) | 46±4 | 40–56 | 37–52 |
| Aspartate aminotransferase (μkat/l) | 0 28±0 9 | 0 11–0 40 | 0 20–0 70 |
| ALAT (μkat/l) | 0 29±0 17 | 0 08–0 68 | 0 10–0 70 |
| Alkaline phosphatase (μkat/l) | 10±7 6 | 2 3–30 0 | 0 8–4 0 |
| Haptoglobin (g/l) | 2 2±1 0 | 0 6–4 5 | 0 4–2 5 |
| IgG (g/l) | 12 3±3 3 | 7 6–20 0 | 7 0–15 0 |
| IgA (g/l) | 1 4±0 4 | 0 6–2 0 | 0 8–3 8 |
| IgM (g/l) | 1 2±0 3 | 0 8–1 9 | 0 4–2 0 |

in males and the IgA is usually higher in older than in younger patients (11) The longer duration of symptoms recorded in the older patients may be ascribed to a slower tumor growth at advanced age Furthermore, a longer duration of symptoms was correlated to less malignant tumors Age was not correlated to tumor size or location or to the histologic findings The higher values of S ALAT in older than younger patients cannot be explained on the basis of the data analyzed in this study

That the prognosis is more favorable in the case of distally than proximally located tumors has been suggested in earlier reports (5 7) In the present series distal tumors were significantly smaller than proximal The initial serum levels of Hb albumin and haptoglobin and the ESR in these patients were indicative of a less advanced stage of the disease Herein may lie an explanation for a reported better prognosis for patients with distally than proximally located tumors (7) especially as we found no correlation with the tumor pathology It would be interesting to see these relationships analyzed in other patient series

The tumors of fibroblastic type were of a lower grade and more common in the female than the male patients Five of the 10 patients followed up for more than 5 years are surviving 4 of them had tumors of the fibroblastic type

The fact that alkaline phosphatase activity varies with skeletal growth complicates any evaluation of recorded levels of this enzyme especially in osteosarcoma the onset of which often coincides with the growth spurt even if the expected growth rate is not exceeded (3)

In the present series the enzyme activity varied widely irrespective of age and the mean was twice the reference value This variable was not correlated with any of the other blood variables or with the prognostic factors studied in these series The alkaline phosphatase activity has been regarded as a valuable index of tumor growth in previous reports (9 12 13) but probably the age of the subjects has not been considered in the analyses The use of reference values for such analyses can also be questioned (6 15)

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## REFERENCES

1 Adamson U Aparisi T Brostrom L Å Cantell K Einhorn S Hall K Ingimarsson S Nilsonne U Strander H & Soderberg G Interferon treatment of human osteosarcoma Study week of the Pontificial Academy of Sciences Vatican City Oct 17–21 1977 In press 1979

2 Broders A C Hargrave R & Meyerding H W Pathological features of soft tissue fibrosarcoma Surg Gynecol Obstet 69 267 1939

3 Brostrom L Å Adamson U Filipsson R & Hall K Longitudinal growth and dental development in osteosarcoma patients Acta Orthop Scand In press 1980

4 Brostrom L Å Aparisi T Ingimarsson S Lagergren C Nilsonne U Strander H & Soderberg G Can historical controls be used in current clinical trials in osteosarcoma? I Analysis of prognostic factors in a historical and a contemporary group J Rad Oncol Biol Phys In press 1979

5 Cancer Treatment Reports Proceedings of the Osteosarcoma Study Group Meeting Feb 1978 National Cancer Institute NIH USA Cancer Treat Rep 62 187 1978

6 Cohen P Osteosarcoma of the long bones Clinical observations and experiences in the Netherlands Eur J Cancer 14 995 1978

7 Dahlin D C Bone tumors—general aspects and data on 3 987 cases 2nd ed 3rd printing p 156 Thomas Springfield Ill 1973

8 — Pathology of osteosarcoma Clin Orthop 111 23 1975

9 Franseen C C & McLean R The phosphatase activity of tissues and plasma in tumors of bone Am J Cancer 24 299 1935

10 Lockshin M D & Higgins I T T Prognosis in osteogenic sarcoma Clin Orthop 58 85 1968

11 Merler E & Rosen F The gammaglobulins N Engl J Med 275 480 1966

12 Scranton P E Jr DeCicco F A Totten R S & Yunis E J Prognostic factors in osteosarcoma Cancer 36 2179 1975

13 Skrovina B Cervenansky J Kossey P & Brozmanova E Primary osteosarcoma A clinical evaluation of 73 cases Neoplasma 184 377 1971

14 Strander H Cantell K Ingimarsson S Jacobsson P Å Nilsonne U & Soderberg G Interferon treatment of osteogenic sarcoma—a clinical trial Conference on "Modulation of host immune resistance in the prevention or treatment of induced neoplasias Dec 9–11 1974 Fogarthy Int Center Proc US Government Printing Office Washington DC 28 377 1975

15 Thrope W P Reilly J & Rosenberg S Prognostic significance of alkaline phosphatase measurements in patients with osteogenic sarcoma receiving chemotherapy Cancer 43 2178 1979

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**DEBATE**

# The Established Relationship among Diet, Serum Cholesterol and Coronary Heart Disease

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The following is a response to an article by L. Werkö in this journal (206 435 1979)—its main assertions and major omissions.

1. Werkö asserts without giving any facts that in the incidence data of the U.S. national cooperative Pooling Project on quintiles of serum cholesterol and future CHD risk "Only in the highest quintile is the risk appreciably higher". In fact risk was higher by 18%, 96% and 139% for men in quintiles III, IV and V respectively compared to quintiles I and II combined (98). Absolute excess risk of a first major coronary event before age 65—i.e. a nonfatal myocardial infarction (MI) or an acutely fatal episode of coronary heart disease (CHD)—was 24, 104 and 161 per 1000 for men in quintiles III, IV and V respectively. These three quintiles had serum cholesterol levels of 218–240, 240–268 and 268+ mg/dl. Clearly at least 40% of these men—quintiles IV and V, and not just quintile V—were at markedly increased risk, i.e. double or greater. Nor is the excess risk of quintile III—greater by 18%, 24 extra chances per 1000 of premature major CHD—to be dismissed lightly.

This experience of Pooling Project men with serum cholesterol of 218 or over, extrapolated to the U.S. male population age 40–64 in 1980, with about 25 000 000 men free of CHD (i.e. like the Pooling Project cohort), would mean almost 1.5 million excess major coronary events before age 65. About 44% of these events would be in quintiles III and IV, the other 56% in quintile V of serum cholesterol. With about 44% of these first major coronary events terminating in acute fatalities (12), *excess deaths* before age 65 would number about 636 000, about 280 000 of them in quintiles III and IV. These are—it is relevant to emphasize—*excess* CHD events and deaths before age 65 attributable to hypercholesterolemia, i.e. events and deaths over and above those to be anticipated if all 25 million of these men had serum cholesterol levels under 218 mg/dl. Particularly since Werkö expresses concern about presumed economic consequences of dietary efforts to prevent CHD, it is relevant to note the huge costs that would accrue from these hundreds of thousands of excess events and deaths—direct costs in medical care and indirect costs in losses to production, together amounting to billions of dollars.

Fortunately, there have been improvements (modest to date) in the eating patterns of Americans and associated declines in population mean serum cholesterol levels, together with large scale cessation of smoking and major advances in the control of hypertension (42, 117, 119–121). Concomitantly U.S. mortality rates from premature CHD have been declining steadily, e.g. by about 25% from 1968 to 1977 for men, in contrast to the rising rates in several northern European countries (e.g. up by 16% for Swedish men age 55–64) (14, 47, 91, 117, 119–121). Hence the outlook for American men in the 1980s is less grim than would be anticipated from the experience of the Pooling Project cohort in the 1950s and 1960s.

2. Werkö asserts that risk of MI for men in the Gothenburg study "is about the same for the four lowest quintiles of cholesterol and appreciably higher only for the highest quintile". No reference is given. The published facts available to this writer—in a paper co-authored by Werkö—show a markedly higher risk for over 50% of men, not just 20% (130). Thus for incidence of nonfatal MI plus fatal CHD in 10 years, with 198 to 216 men in each of four serum cholesterol classes (under 215, 215–241, 242–271, 271+), numbers of events were 4, 18 and 12 respectively (all m… numbers of CHD deaths …

Clearly both stratum III and IV h d much higher rates—risk ratios of 3 7 and 2 5 for CHD incidence compared to strata I and II 16 7 and 14 8 for CHD death

3 In discussing U S studies on serum cholesterol and CHD risk Werko asserts their results do not always check with those from more carefully conducted European studies He gives no criteria for this judgment about rese rch quality and cites no references This writer is aware of 32 prospective epidemiologic studies that have presented data on serum cholesterol and CHD risk for the individuals of their cohorts—13 in the U S 15 in 11 European countries 4 elsewhere (Australia Israel Japan Puerto Rico) (12 15 28 30 31 34 35 39 40 41 43 45 49 52 54 55 60 61 64 65 68 75 78 80 81 83 87 93 96 98–100 103–108 113 115 119 122 125–127 130 131 134 138 145) Of these 32 28 found serum cholesterol at baseline significantly related to CHD risk A majority of these studies also did multivariate an lyses demonstrating in most instances—among them the Gothenburg study (138)—that the relationship of cholesterol to CHD risk is a significant independent one Of the four studies not showing a statistic lly significant relationship ($p \leq 0.05$) (43 83 100 113) three trend in that direction One of these (the Tromso study) is based on only two ye rs of follow up with only 11 first coronary events A second (the Glostrup study) has a small sample size (375 men) with 29 first CHD events in 10 years In regard to these studies one should avoid inter ig a statistically nonsignificant difference as proof that no real difference exists (48) Overall the findings of this large number of prospective studies across the world are remarkable for their consistency including consistency within and between the American and European studies This is particularly noteworthy in view of the fact that most or all of these studies classified their cohorts at entry based on only one cholesterol measurement with its known limitations due both to intra individual variability and laboratory error The positive findings demonstrating a relationship of serum cholesterol to CHD incidence are of a scope and consistency rare in medical research on chronic non infectious diseases

4 With regard to the data from the U S Pooling Project, Werko asserts Framingham is the only part based on a population which has enough end points to make an analysis possible The Final Report of the Pooling Project presents analyses sep rately for each of the eight studies as well as the combined data for the five ppropriately poolec (98) Numbers of first m jor coronary events were 162 123 142 181 and 50 respectively for these five (Alb ny Chicago G s Co Chicago Western Electric Co Fr mingham Tecumseh) 72 29 nd 113 for the Los Angeles Minnesota business and professional men and northwest railroad workers respectively Numbers of events and CHD deaths were lso sizeable for the men ige 40–54 in the five pooled studies e g 65 44 61 59 and 27 CHD deaths (78) Clearly these numbers do not support Werko s assertions His allegation on this matter is p rticularly puzzling given the fact that he apparently deemed it appropri te to an lyze and report data from the Gotenburg study based on 39 incidence events with 18 CHD deaths (130)

5 Werko also asserts Stamler now uses the NPP (Pooling Project) in his main argument after his own study of the Chic go G s employees has demonstrated that there is very little difference in CHD incidence in relation to the original serum cholesterol value The facts on the Peoples Gas Co study are With 9 7 years of follow up (as used in the Pooling Project) of the cohort of 126 white men ige 40–59 at entry in 1958 risk ratio for serum cholesterol quintile V is 1 5 for quintile IV 1 6 (98) (For similar d t with 12 year follow up see reference 122) For the 936 white men age 40–54 at entry in 1958 gain with 9 7 years of follow up *t* values for serum cholesterol and CHD incidence in univari te and multivariate analyses are 2 60 and 2 06 respectively ($p<0.01$ and $<0.05$) (78)

The writer has also shared responsibility for years for two other Chicago prospective studies i e the Western Electric Co (78 98 125) and the Chic go Heart Association Detection Project in Industry studies (126) In multivariate an lyses both of these show a strong significant independent association between baseline serum cholesterol and CHD

6 One of Werko s m in assertions is that non response—the refusal to p rticipate by a proportion of the population—introduces bias into prospective epidemiologic studies He implies th t this neg tes the validity of their findings on risk factors and their gener lizability to the population at large It is most unlikely that Werko s hypothesis has any relation to the facts Thus in terms of serum cholesterol and CHD his hypothesis requires th t in the

non responders there would be a very strong *inverse* relationship between serum cholesterol and CHD risk such that the direct relation observed in the responders would be cancelled out if the non responders were in the analysis There is no evidence to support this hypothesis and no reason to believe that in non responders—in conspicuous contrast with responders—serum cholesterol is inversely related to CHD risk

7 Werko asserts Another corner stone in the lipid heart theory is the straight line relation between serum cholesterol and the later incidence of clinical manifestations of CHD Among other references he cites a recent review by this writer to document this point although careful reading reveals that this concept is *not* set forth anywhere in that paper (119) In fact much of the curve fitting of data on serum cholesterol and CHD has shown a good fit with the multiple logistic model i e a curvilinear—not a straight line—relationship Cornfield (23) in 1962 published an analysis indicating that the relationship is curvilinear i e exponential CHD incidence=1 (1 serum cholesterol)[2 66]

The exact shape of the curve is of secondary importance The primary issue is whether the association between serum cholesterol and CHD risk is *etiologically significant* Since the 1964 *Report to the Surgeon General on Smoking and Health* (1) clear criteria have been readily available to assess whether epidemiologic associations reflect cause and effect relationship In updated form they are a *strength* of the association b *graded* nature of the association c *temporal sequence* i e does the presumed etiologic factor precede the disease d *consistency* of the findings in multiple studies in different populations e *independence* of the association i e does the presumed factor relate to the disease after accounting for other variables associated with the disease f *predictive capacity* i e ability based on the findings in one or more sets of populations to predict events in other different populations g *coherence* of the findings—in two senses i e consistency of the epidemiologic findings with those for other research methods (animal experimentation clinical and pathologic investigation) and coherence in the sense that reasonable pathogenetic mechanisms have been identified whereby an etiologic agent acts to produce (or help produce) the disease (16)

Note that a linear relationship is not among the criteria In fact a trait may have a quadratic (U shaped) relation to CHD incidence and may be an etiologically significant risk factor (32)

The evidence has been summarized above on *temporal sequence consistency* and *independence* of the relationship between serum cholesterol and CHD risk Some aspects of the *coherence* criterion are briefly presented later in these comments including key findings from animal experimental short term metabolic ward and long term intervention studies Since it has been shown repeatedly that dietary cholesterol fat influences serum cholesterol β lipoprotein distributions and levels that β lipoprotein (LDL) enters intima and is atherogenic that accumulation of excess cholesterol and especially esterfied cholesterol is the hall mark of the atherosclerotic plaque and that severe atherosclerosis is the underlying pathologic process in most (90+%) cases of clinical CHD coherence in terms of a reasonable pathogenetic mechanism is hardly an issue As to the criterion of *predictive capacity* since Keys first successfully applied the Framingham multiple logistic coefficients (including the coefficient for serum cholesterol) to predict CHD risk in the cohorts of the Seven Countries Study (60) this has been done repeatedly—e g in two comprehensive reports on the populations in the Pooling Project (78 98) See also our group s 1972 review (122) and the article by Keys Predicting Coronary Heart Disease in the monograph edited by Tibblin Keys and Werko (61) As to the *strength* of the relationship reference to data from the U S Pooling Project and the Gothenburg study are representative In the former risk ratios for a first major coronary event for men in the fifth quintile of serum cholesterol (>268 mg/dl) compared to those in the first and second quintiles combined (≤218 mg/dl) are 3 6 at age 45–49 2 1 at age 50–54 and 55–59 1 5 at age 60–64 2 4 overall (approximate 95% confidence interval 1 9–2 9) (98) Similar data are given in 2 above for the Gothenburg study of men age 50 at entry followed for 10 years (130) Clearly the relationship is strong—not a 10% or 20% increase in risk but a more than doubling or tripling of risk of a nonfatal MI or fatal CHD before age 60

As to the criterion *graded nature* of the relationship Werko implies it is not met in either the Gothenburg or Pooling Project studies The actual findings of the two studies in this regard have already been presented in 1 and 2 above In the

several prospective studies were univariate regression analyses have been done good correspondence has repeatedly been registered between expected and observed CHD rates in quintiles of baseline serum cholesterol with a clearcut graded relationship Where multiple logistic analyses have been done including in the Pooling Project and Gothenburg studies the coefficients for serum cholesterol in most cases have $t$ values of 2 00 or greater indicating a statistically significant consistent independent strong graded relationship between serum cholesterol and CHD incidence (see 3 above for references)

Further as to the criterion of graded relationship (*i e the more severe the hypercholesterolemia the* greater the risk) there is the experiment of nature well known to clinicians for almost 100 years originally known as essential familial xanthomatosis (56) now more precisely characterized as genetic hyperbetalipoproteinemia With the severe hypercholesterolemia hyperbetalipoproteinemia present from infancy on victims of this genetic defect often succumb to severe coronary atherosclerosis and consequent coronary heart disease early in life even in the second decade for homozygotes

In brief extensive data sets from many sources and of many types document that serum cholesterol is related to CHD incidence in an etiologically significant way the findings from these data sets meet all seven criteria indicative of a causative relationship

8 Werko asserts that in its Final Report the Pooling Project shows that the standard incidence ratio in all studies and in pool 5 was higher in the lowest quintile of serum cholesterol than in the next The facts are For Pool 5 with 86 and 80 first major coronary events in quintile I and II respectively *standardized incidence ratios (SIR) are* 72 and 61 (98) In four of the eight studies the SIR is lower in quintile I than in quintile II in the other four higher For Pool 5 for the age groups 40–44 45–49 and 60–64 the SIR is lower in quintile I than II for age groups 50–54 and 55–59 higher Thus the findings are inconsistent They do not permit a definite judgment as to whether middle aged American men with serum cholesterol levels <194 mg/dl (quintile I) are at lower risk of CHD than those in the range 194–218 (quintile II) Clearly there is need for more research on this matter as pointed out in the Pooling Project Final Report (98) That is all that can be soundly concluded from these data They are not—as Werko implies—a refutation of the conclusion that elevated serum cholesterol is an etiologically significant major risk factor for CHD

9 Werko also asserts about the Pooling Project Final Report quite a lot of statistical sophistication is used to analyze the combined feature myocardial infarction fatal and nonfatal plus sudden coronary death This end point is the commonly used measure of hard coronary incidence i e incidence of coronary events—nonfatal MI and fatal CHD—most amenable to standardized objective measurement (unlike angina pectoris and congestive heart failure for example) It was an original key end point identified by the Pooling Project Research Group from the beginning reported on in both preliminary and final communications (12 49 78 98) It is an end point used in virtually all prospective epidemiologic studies throughout the world including the Gothenburg study (130)

10 Werko also makes the following assertions about the Pooling Project findings on serum cholesterol and CHD mortality serum cholesterol values bear no straight line correlation to the occurrence of sudden death within 10 years The argument that dietary changes should postpone sudden death has no background even in the best and often quoted American study The total lack of correlation between sudden death and serum cholesterol in the preliminary report led the authors to omit this analysis in the final report The facts are In the initial analysis on sudden death for men age 30–59 at entry in serum cholesterol intervals 250 mg/dl age adjusted 10-year mortality rates from sudden death are in the range 11–16/1000 for those in the intervals 250+ the rates are 25–30/1000 (12 49) For the 32% of the men with serum cholesterol 250+ mg/dl the risk ratio for sudden death is 2 0 *compared to those <250 This is not a* total lack of correlation

While the sudden death end point has been a focus of interest in the 1970s overall acute mortality (sudden and non sudden) with first major coronary events is also of great importance Thus in the Pooling Project cohort about 60% of deaths with first major coronary events were sudden (<3 hours from onset of symptoms) 40% non sudden (≥3 hours to one month with many of these in the period 3–24 hours) For both the sudden and non sudden deaths a sizeable per cent occur so rapidly that patients do not reach a hospital—about 70% overall (122) Clearly then the end point of

er ll CHD mortality (sudden and non sudden) w th f rst e ents s a very mportant one The pre l m nary data of the Pool ng Project on th s end po nt are age adjusted mortal ty rates n the range ?? 8/1 000 for those n the four lower cholesterol intervals (<?50 mg/dl) 41 55/1 000 for those n the three h gher intervals (250+) w th an overall risk rat o of ? 0 aga n i e the same rat o as for the sudden death end point by itself

CHD mortal ty data have also been publ shed in f nal form for the f ve stud es mak ng up Pool 5 (78) For each of them mean serum cholesterol was h gher—by 1? to 44 mg/dl— n cases of fatal CHD than n non-cases For four of the f ve stud es the $p$ value for serum cholesterol and CHD death was <0 05 for one (the Ch ago Peoples Gas Co ) >0 05 <0 10 (cf also ref 1??) In the mult ple log st c analyses w th systol c pressure and c ga rette smok ng also cons dered $p$ value was <0 001 for one study >0 05 <0 10 for three others

In a separate analys s done for the cohort of 1 595 Western Electric Co men age 4? 58 in 1960 the $p$ value for serum cholesterol and CHD death n a 6-factor mult ple log stic analys s s <0 05 (follow up period is 15 years) (1?5) For 6493 wh te men orig nally age 40–59 n the Ch cago Heart Assoc a tion Detect on Project n Industry study w th 5 year follow up this $p$ value n the mult ple log st c analys s s <0 001 (1 6)

11 In regard to persons recovered from myocar d al nfarct on Werko asserts D et or blood l p ds are of no consequence for the f nal outcome of these pat ents In fact the data on the ?789 men in the placebo group of the Co onary Drug Project clearly show that serum cholesterol is s gn f cantly related to long term r sk after recovery from MI (?6) Th s s the cons stent f nd ng n un variate b variate (w th fast ng serum triglycerides) and 40-factor mul t variate analyses for four end po nts (nonfatal re current MI plus fatal CHD sudden CHD death all CHD death death from all causes) Thus hyper cholesterolem a along w th c garette smok ng (as correctly noted by Werko) is one of few post MI risk factors amenable to safe mod f cat on (by d e tary means n the case of hypercholesterolem a) The nference s that a s zeable potent al ex sts to reduce absolute risk for these very h gh risk pa ents The encourag ng data from two secondary pre ent on trials nvol ng serum cholesterol con trol by d etary means lend support to th s nference (53 0

1? In regard to the results of trials Werko speaks of The repeated demonstrat on (that) man pulat on of the serum cholesterol value in man does not influence the mortal ty rate He c tes two primary pre ention trials—the 3-center European clof brate trial and the F nnish Mental Hosp tal Study (8? 93) An extens ve discuss on of the de ta led f nd ngs reported from the clof brate trial s not n order here but two key points are relevant F rst data from the h gh versus the low cholesterol control group (both g ven placebo) aga n demon strate the relation of serum cholesterol to CHD risk nclud ng risk of CHD death The risk rat os for the h gh versus the low cholesterol control group based on age standard zed rates for men age 40–59 at entry are for all major schem c heart disease (IHD)—? 7 for nonfatal MI—? 9 for fatal IHD—? 3 for sudden IHD death—? 0 Werko does not ment on these results Second the clof brate trial was a dr g— ot a d et—trial L ke all other drugs clof brate used long term enta ls risks as both the European and U S trials showed (?4 25 93) Therefore failure of a drug trial to reg ster reduct on n CHD or all causes mortal ty cannot be used to draw nferences about ab l ty to prevent CHD by safe d etary measures No generalization about diet is scient fically sound from the negative or harmful results of a drug trial

As to the F nn sh Mental Hosp tal Study in t al and comprehens ve reports have been publ shed from th s trial of a fat mod f ed cholesterol lowering d et (53 13? 133) It used a cross over des gn in two mental hosp tals each serv ng for alternate 6-year periods as experimental and control s tes It nvol ed 676 men or g nally age 34–64 free of ECG ev dence of CHD at entry W th average follow up of about 4 4 years per man inc dence rates of major ECG change or CHD death n the d et treated and control groups are 4 ? vs 1? 7 per 1 000 man years i e a reduct on n CHD ncidence of 66 9% ($p$ 0 001) For CHD deaths the rates are 3 0 and 6 1 respect vely i e a reduct on n mortal ty of 50 8% ($p$ 0 10) Contrary to Werko s assert on the authors state It s concluded that the use of the serum cholesterol lowering d et exerted a substant al pre ent ve effect on CHD (133)

S m lar results were reported by the Los Angeles Veterans Adm n strat on dom c l ary fac l ty d etary trial the one long term (8+ years) study nvolv ng both random zat on and a double bl nd des gn (?9) Werko makes no reference to these f nd ngs In

brief of the 422 men in the control and 424 in the experimental group (average age 65 5 years at entry) 70 in the control and 48 in the experimental group experienced a fatal atherosclerotic outcome i e a reduction of 31 4% in the diet treated group ($p<0.05$) For all hard atherosclerotic disease end points nonfatal and fatal life table analyses revealed a significant difference in favor of the diet treated group overall ($p=0.02$) Sudden deaths numbered 27 and 18 in the two groups i e 33% lower in the diet treated group

As discussed in detail elsewhere (49 116–119) these and other first generation dietary trials organized in the late 1950s with scant resources had basic design limitations These included in some instances lack of a randomly assigned control group and in all studies sample sizes that were far too small All involved less than 1000 participants but the smallest sample size estimate for a single factor primary prevention trial on diet is 47000 high risk middle aged men (90) Such numbers are necessary to have a reasonable assurance of avoiding both a false negative result and a positive trend that is equivocal Given this fact it is understandable that in some respects the findings of the first generation trials are not definitive e g in regard to mortality from all causes No single factor large scale primary prevention trial on diet and CHD is currently in progress planned or even contemplated in the U S Europe or elsewhere It is all the more important therefore to evaluate the early small ones as objectively as possible Both types of one sidedness must be avoided First it is unsound to be unduly negative i e to fail to note statistically significant differences in favor of diet treatment and to interpret nonsignificant differences as proof that no real difference exists Werko s statements about the trials have these characteristics Second it is unsound to overlook the limitations of these trials and to conclude that they yielded unequivocal results It is this writer s judgment that the assessment in the 1970 Report of the Inter Society Commission on Heart Disease Resources remains balanced and sound The results are encouraging However they are not conclusive Each (study) dealt with a relatively small group and had one or another additional flaw Nonetheless all are consistent in showing a reduction in CHD incidence and indicate the potential for CHD prevention through modification of dietary fat composition (49)

It is necessary also to maintain a balanced view on the importance of data from trials in evaluating both the etiologic role of rich diet and diet induced hypercholesterolemia in atherogenesis and the potential contribution of improved nutrition to CHD prevention Almost certainly a definitive unifactor large scale trial on diet and CHD is not going to be done Results of multifactor trials currently in progress in Europe and the U S may well be difficult to interpret in terms of relative impact of change in diet cholesterol and other factors (e g smoking) (119) For this and other reasons such trials cannot yield the definitive proof in this area Rather they furnish pieces of relevant data that must be evaluated along with the totality of findings from all methodologies

13 Having repeatedly asserted that serum cholesterol bears no relation to CHD death especially sudden unexpected death Werko the ... tly dismisses a strategy of primary prevention against CHD and emphasizes secondary prevention with $\beta$ blockers One of his arguments is about 2/3 of these (sudden) deaths have already had some earlier manifestation of the heart disorder mostly a recognized myocardial infarction No reference is cited This is not the finding in U S prospective studies Thus in ten years of follow up of the entire Peoples Gas Co cohort of 1465 middle aged men the majority of sudden deaths were in men free of any evidence of CHD at entry and were the first manifestation of the disease (122) Other studies have reported similar results

As already noted 44% of all first major coronary events in the middle aged men of the Pooling Project cohort terminated fatally within one month with 61% of these deaths sudden (<3 hours) (12 This is in accord with world wide experience Given these basic characteristics of coronary heart disease only a strategy emphasizing primary prevention can bring this epidemic under control (49 Implementation of this strategy must proceed from childhood on It must be based on all the knowledge concerning the multifactorial causation of the underlying disease severe atherosclerosis of coronary arteries That is its emphasis must be nutritional and hygienic aimed primarily at establishing better life styles in regard to eating drinking smoking exercising

Werko s argument in this area is flawed in yet another aspect He asserts Death—especially sudden unexpected— is due to sudden increas

in catecholamine influence on the heart But as he noted in his previous paragraph The myocardium especially under ischemic conditions may be very sensitive to catecholamines Thus the central problem is coronary ischemia and the strategic key to its prevention in most cases is the primary prevention of severe coronary atherosclerosis Without ischemia secondary to severe atherosclerosis the threat of fatal sudden death from adrenergic influences becomes minor Thus women with less coronary atherosclerosis throughout middle age than men have much lower rates of CHD death including sudden death in the U S A the countries of northern Europe etc

As to $\beta$ blockers for secondary prevention Werko asserts It has been shown that at least some $\beta$ adrenergic blockers diminish the incidence of sudden death after myocardial infraction He speaks of clear cut results with these *medicines the demonstration that $\beta$* blocking agents or at least alprenolol and practolol do decrease the incidence of sudden death in patients after myocardial infarction and coronary deaths in patients with moderate hypertension He cites one reference (139) This is not the place for a detailed critique of the few trials reported to date on long term treatment with $\beta$ blockers but three points are relevant here First it proved necessary to withdraw practolol from use because of serious toxicity

Second Werko s categorical and unequivocal conclusion about the efficacy of $\beta$ blockers—based on only one or two trials—seems to reflect a different approach to accepting evidence on drugs compared for example to his sceptical assessment of the consistent data from the many prospective studies on serum cholesterol and CHD risk Is it not reasonable to ask Is Werko applying a double standard? Is he manifesting bias in his judgments? Might it not be more sound to be more circumspect and guarded about the benefit to risk ratio of $\beta$ blockers for long term treatment post MI at least until the results are available from large scale trials currently in progress in Europe and the U S ?

Third even if improved treatment post MI reduces 5 year mortality by as much as 50% a major achievement indeed risk of death would still be considerably worse after infarct than for persons of the same age and sex without such manifest CHD Thus even such a substantial achievement in secondary prevention is at most a limited and incomplete solution Any focus on it as the main or sole strategic approach—instead of primary prevention—is bound to fail to control the epidemic given the toll with first major coronary episodes

14 Werko states that his purpose is to look a little closer on the evidence favoring the diet heart theory However he attempts no real review of the literature Instead he deals in some detail with only four research reports in this area—from Malmros the Pooling Project the Finnish Mental Hospital Study and the European Clofibrate Trial (73 82 93 98) A few comments are necessary here about Werko s approach to the first of these four Werko alleges Malmros is resting his whole case on the parallelism between mortality from arteriosclerosis and fat consumption in each of these countries In fact Malmros is too intelligent knowledgeable and experienced a scientist to rest his case on one piece of research evidence That is *undoubtedly why Malmros also devoted years to* research in this field including—for example—careful animal experimentation on dietary lipids serum cholesterol and atherogenesis His important contributions include the first production of atherosclerosis exclusively by dietary means in dogs (74)

One of Werko s criticisms of the Malmros paper on wartime trends is he used the crude death rate for arteriosclerotic diseases But Malmros was concerned with *short term* (1935–47) trends of mortality *within* each country For this purpose crude death rates may suffice provided there is no evidence of a substantial change in the age composition of the population that would confound the analysis

Werko also criticizes Malmros and others for linking dietary fat and arteriosclerosis mortality trends during the war while ignoring wartime rationing of cigarettes and gasoline two other commodities that might influence the mortality But do these latter trends if they did indeed play a role negate the possible contribution of the decline in consumption of animal fat?

Werkö also argues that even if the wartime declines in mortality noted by Malmros for Finland Norway and Sweden are valid their most probable explanation is the wartime lack of respiratory epidemics after an influenza epidemic in the late 30s and the advent of anti infectious treatment with the emerging sulfa drugs These Werkö asserts are quite enough

explain the short term decrease in mortality But why then the steady rise—not fall—in the U S A rates during the war years as shown by Malmros and others (73 85 86)? Also with the marked long term declines in influenza-pneumonia death rates in all these countries why the increases in the CHD mortality rates after the war? Why the rise in Sweden from 1969 to 1977?

In fact the analysis of factors possibly responsible for time trends of mortality is one of the most difficult problems in CHD epidemiology (57 85 86) The reason is simple the limited amount of data available both on the disease trends and the factors possibly influencing them the limited precision of the data and no way to go back and expand or improve the data base But nonetheless—as with all data sets—something is to be learned here too from careful consideration of all the facts And an important point that must be made about Werko s treatment of this problem is his focus on one paper only and his ignoring of the many other reports showing a positive relationship between time trends of dietary lipid and of CHD Particularly since Werko cited this writer s recent review (119) it is relevant to note that it included a summary of work in this area encompassing 14 references to original papers or reviews so that the materials were close at hand It is relevant also to note the most recent report on this matter by our own group (14) Among other things it shows for the industrialized countries a statistically significant association between trend of dietary cholesterol (1954–65) and trend of CHD mortality (1969–75) also between trend of dietary meat poultry eggs dairy products and trend of CHD mortality ($p<0.05$) for men for women and for both sexes combined age 35–74 The recent reports on trends in the German Federal Republic are also highly relevant to the issues under discussion (110 111)

15 Werko also fails to mention many other sets of research data contrary to his contentions Thus at least a dozen studies over the last 25 years have consistently shown significant positive high-order correlations between dietary lipids available per person per year for different countries (i e choles terol saturated fat total fat) and the CHD death rates in middle age for these countries (7 19 51 58 66 71 76 119 124 128 146 147) These associations have been repeatedly recorded despite the limited precision of both the dietary and mortality data (from FAO and WHO reports respectively)

16 Werko also ignores the data from international comparisons of autopsy series with findings in accord with those cited in 15 above (57 119) The International Atherosclerosis Project is the most comprehensive study of this type involving 31 000 decedents in 15 locales across the globe two industrialized (Oslo New Orleans) the others non-industrialized in Latin America Africa and the Far East (79) It found marked differences in the extent of severe atherosclerosis (aortic and coronary) among the decedents from different countries and significant positive high-order correlations among population mean dietary fat intake mean serum cholesterol and mean severity of atherosclerosis (Data on dietary saturated fat and cholesterol for the non industrialized countries were too fragmentary to permit analysis they generally paralleled total fat intake )

17 Werko also ignores the international cross-sectional and prospective studies of living population samples yielding similar results Several relevant sets of data of this kind were reported in the 1950s e g showing much lower mean serum cholesterol lipid $\beta$ lipoprotein levels and far lesser increases in these levels from youth to middle age in predominantly vegetarian populations (in nonindustrialized countries of Africa Asia and Latin America in Mediterranean countries and in Japan) compared to populations of northern Europe and the U S (57 119) These latter populations ingest high percentages of calories from animal products with consequent high intakes of saturated fat and cholesterol Upper class strata in the non industrialized countries with eating habits like northern Europeans and Americans have serum lipid patterns like northern Europeans and Americans (56 57 119) These findings together with those on migrants (see below) indicate that the observed differences between countries are not due to differences in population genetics they are environmental—first and foremost nutritional—in origin

The Seven Countries Study is the most comprehensive prospective international investigation of living population samples—18 altogether comprising about 12 000 middle aged men in Finland Greece Italy Japan Netherlands U S and Yugoslavia (64) Both in its 5 and 10-year follow ups data it has recorded significant high-order correlations among group mean saturated fat intake group mean serum cholesterol and CHD incidence rates (60 62) (Dietary cholesterol was not measured )

18 Werko also ignores the several sets of epidemiologic data on migrant populations—e g African and Asian Jews to Israel Neapolitans to Boston Japanese to the U S These further confirm the causative link among diet (particularly lipid composition) serum cholesterol lipid β lipoprotein and CHD (57 119) The Ni Hon San study—involving sizeable samples of middle aged men of Japanese ancestry in Hiroshima and Nagasaki Japan Honolulu Hawaii U S and the San Francisco Bay Area California U S —is a major prospective study of this type (52 55 75 103 104 119 131 145) Diets of the Japanese were strikingly different from the Japanese Americans e g group mean intake of saturated fat 3–4 times greater for the latter dietary cholesterol also greater Mean serum lipids were correspondingly different e g serum cholesterol 181 1 for the Japanese cohort 218 3 and 228 2 for the two Japanese American samples CHD *prevalence incidence and mortality rates differed* accordingly

19 Werko also ignores the reported epidemiologic analyses of populations from different regions ethnic groups religious sects etc within specific countries These too confirm the links *among diet serum lipids lipoproteins and CHD In* his recent review (119) the writer cited 29 references in this area For example one of them presents findings for Americans consuming Japanese type fare low in saturated fat and cholesterol (109) These residents of a Boston commune eating a macrobiotic diet in keeping with their Zen Buddhist views had mean serum total cholesterol LDL and VLDL cholesterol levels 31–38% lower than those of age sex weight matched controls The several reports on Seventh Day Adventists in the U S A compared to other Americans also merit specific mention (119) especially the paper on CHD mortality (97) The recent report on different regions in Belgium (67) is a meaningful addition to the cited list (119)

20 Werko also ignores the data from several clinical investigations on serum cholesterol level and severity of coronary atherosclerosis as demonstrated angiographically *In the 1970s at least* eight reports were published on this matter all consistently showing a positive relationship (13 16 17 27 37 38 69 135)

21 Werko also ignores the extensive metabolic ward investigations of samples of healthy adults demonstrating in detail the influences of dietary lipid (cholesterol saturated and polyunsaturated fat) on serum cholesterol under isocaloric conditions—e g 63 experiments by Anderson Grande and Keys (63) 36 by Hegsted and colleagues (44) several by Ahrens et al (3) Mattson et al (33 77) and Connor et al (19–22)

In addition there are the recent findings of Mahley and colleagues showing that feeding eggs (i e cholesterol) induces changes—possibly atherogenic—in the distributions of lipoprotein fractions and subfractions even in those persons manifesting no increase in serum total cholesterol (72)

22 Further although the theme Werko addresses in his opening sentence is the diet heart theory or lipid heart disease theory Werko also fails to cite and discuss the many long term intervention studies showing that sustained decrease in saturated fat and cholesterol intake produces sustained fall in serum cholesterol He cites only the Finnish *Mental Hospital Study erroneously states that its* last report was in 1972 and does not discuss it substantively He totally ignores the results of other investigations on the influence of diet on serum lipids—e g the Faribault Minnesota mental hospital study (90) the Los Angeles Veterans Administ*ration domiciliary facility study (29) the Chicago* Coronary Prevention Evaluation Program (115–118) the Multiple Risk Factor Intervention Trial (MRFIT) (88) the National Diet Heart Study (90) the New York Anti Coronary Club (102) the Oslo primary and secondary prevention trials (46 70) In this way Werko avoids discussing the significance of the relationships repeatedly demonstrated in man among diet serum lipids lipoproteins and CHD

23 Werko ignores completely the thousands of experiments reported over the last 70 years—since the first production of atherosclerosis in animals in Russia in 1909 (6) These have repeatedly shown that sustained feeding of diets containing increased quantities of cholesterol and fat is a virtual prerequisite for the production of significant atherosclerosis in a wide range of species studied in the laboratory including non human primates (6 *56 57 95 112 115 142)* Animal experimentation has encompassed feeding small amounts of cholesterol to rabbits chickens and monkeys at levels present in usual human diets in western industrialized countries Although little or no hypercholesterolemia supervened atherosclerotic lesions developed nonetheless (6 8 9 56 57 123) In the

Superintendent of Documents U S Go ernment Print ng Off'ce Wash ng on D C 1964
2 Ad sory Panel of the Comm ttee on Med cal As pects of Food Fol cy (Nutr on D et and cor nary heart d sease Departn nt of Heal h and Secur ty Her Majesty s Stat onery Off ce London 1974
3 Ah ens E H Jr Insull W Blomstrand R Hirsch J Tsaltas T T & Peterson M L Lancet 1 943 953 1957
4 American Heal h Found t on Prev Med 1 255 86 1972
5 American Heart As oc at on Centr l C mm ttee for Med cal and Commun ty Prog am D etary fat and ts relat on to heart attacks and strokes American Heart Assoc a on New York EM 180 1961
6 An tschkow N Exper mental arter o cle os s n an mals In Arter osclero s (ed E V Cowdry) pp 271 322 Macm llan New Yo k 1933
7 Armstrong B K Mann J I Adelst n A M & Esk n F J Chron D s 8 455–469 1975
8 Arm trong M L Regress on of a heroscl ros s In Atherosc eros s rev ews vol 1 (ed R Paoletti & A M Go to Jr) pp 137 182 Ra en Press New Yo k 1976
9 Arm trong M L Megan M B & Warner E D C c Res 34 447–454 1974
10 Austral an Academy of Sc en e D et and coronary heart d ease Report no 18 G ll n Pre s Netley South Austral a 1975
11 Blackburn H D et and m ss hyperl p dem publ c health consequences—a po nt f ew In Nutr t on l p ds and corona y heart d ea e—A global v ew (ed Levy et al ) pp 309 348 Ra en Pre s New York 1979
12 Blackburn H Chapman J M Dawber T R et al Am Heart J 94 539 540 1977
13 Blo h A D nsmore R E & Lees R S Lancet 1 9 8 1976
14 By ng on R Dye A R Gars de D L u K Mo D Stam e J & T ong Y Recent trends of majo co onary r sk fact rs and CHD mortal ty n the U n d Sta e and her ndu r l zed countr es In P oceed ng of the Confe ence n the Decl ne n C onary Heart D sease Mortal ty ed R J Ha l k and M Fe nle b) pp 340–379 U S Depart ment of Health Educat on and Welfare Publ c Health Ser ce Nat onal In t tutes of Health NIH Publ cat on 9 1610 1979
15 Carl on L A & Bo t ger L E Lancet 1 865–868 1972
16 Cohn P F Go l n R Vokona l S W ll ams R A & Herman M V N Engl J Med 86 901 907 197
17 Cohn P F Gabbay S I & Wegl k W B Ann Intern Med 84 241 45 1976
18 Comm ttee on D et and Heart D ea e Nat onal Heart Foundat on of Au ral a Med J Aust 1 575 579 616–6 0 663–668 1974
19 Connor W F Geriatrics 16 407–415 1961
20 Connor W E Hodges R E & Ble ler R E J Lab Cl n Med 57 331 342 196
21 — J Cl n Invest 40 894–901 1961
22 Connor W E S one D B & Hodges R L J Cl n In est 43 1691 1696 1964
23 Cornfeld J Fed Proc 21 58–61 1962
24 C ronary Dr g Pr ject Research G oup JAMA 231 360–381 1975
25 — N Engl J Med 296 1185 1190 1977
26 — Am J Ca d ol 42 489–498 1978
27 Crowley L V Cl n Chem 17 06– 09 1971
28 Cullen K J Med J Aust 714–718 1972
29 Dayton S Pea ce M L Ha h mot S D xon W J & T m yasu U C rculat on (Suppl) 2 19 9
30 Duc met ere P E chwege E R chard J Claude J & Ilgr shi I J Chron D s 32 759 66 1979
31 Duc met e e P Warnet J M & R ha d J L J Chron c D s 9 423–4 9 19 6
32 Dyer A R Stamler J Berkson D M & L ndberg H A J Chron c D s 8 109 123 1975
33 E ckson B A Coots R H Mattson F H & Kl gm an A M J Cl n Invest 43 017 025 1964
34 Friedma G D Klat ky A L S egelaub A B & McCarthy N Am J E p dem ol 99 101 116 1974
35 Fuller J H M Cartney P Jarrett R J et al J Cl on c D s 32 721 7 8 1979
36 Ge man S c ety for Nutr t n The nutr t on report 1972
37 Gottl eb C Fre s nger F Page E E & Ro s R S Trans Assoc Am Phys c ans 83 8–90 19 0
38 Gotto A M Gorry G A Thomp on J R et al C rculat on 56 875–883 1977
39 G ldbourt U Medal e J H & Neufeld H N J Chron c D s 8 17 37 1975
40 Go don T Garc a Palm eri M R Kagan A Kannel W B & Sch ffm an J J Chron c D s 27 3 9 344 1974
41 Grabau kas W & Glazunov I Personal commun cat on 1979
42 Havl k R J & Fe nle b M (ed ) Proceed ngs of the Conference on the Decl ne in Coronary Heart D s ea e Mortal y U S Department of Heal h Educa t on and Welfa e Publ c Health Serv ce Nat onal Inst tutes of Health NIH Publ at on no 79-1610 Wash ngton D C 1979
43 Haw h rne V M & G lmour W H J Chron c D s 32 787 736 1979
44 Heg ted D M McGandy R B Myers M L & Stare F J Am J Cl n Nutr 17 281 95 1965
45 Heyman A Ka p H R Heyden S et al Arch Intern Med 28 949 955 1971
46 Hjermann I The Oslo Study—smok ng l p d pri mary pre ent on tr al in men aged 40–49 In Ischae m c heart d ease (ed A T Hansen P Schnohr and G Rose) p 162 FADL s Forlag Copenh gen 1977
47 Hunter J D et al (Sc ent f c Comm tee of the National Heart Foundation of New Zealand) C ro nary heart d ease—*A New Zealand report* Nat onal Heart Foundat on of New Zealand Sept 1971
48 Hypertens on Detect on and Follow Up Progra Cooperat e G oup JAMA 242 2562 2571 1979
49 Inter Soc ety Comm ss on for Heart D ea e Re sources Atheroscleros s Study Group and Ep dem ology Study Group C rculat on 42 A55 1970

50 Joint Working Party Royal College of Physicians of London and the British Cardiac Society J R Coll Physicians Lond 10 214–276 1976
51 Jolliffe N & Archer M J Chronic Dis 9 636–652 1959
52 Kagan A Harris B R Winkelstein W Jr et al J Chronic Dis 27 345–364 1974
53 Kallio V Hamamainen H Hakkila J & Luurila O Lancet 2 1091–1094 1979
54 Kannel W B & Gordon T The Framingham Study An epidemiological investigation of cardiovascular disease Sect 30 Some characteristics related to the incidence of cardiovascular disease and death Framingham Study 18 year follow up U S Department of Health Education and Welfare Public Health Service National Institutes of Health DHEW Publication no (NIH) 74-599 Feb 1974
55 Kato H Tillotson J Nichaman M Z Rhoads G G & Hamilton H B Am J Epidemiol 97 372–385 1973
56 Katz L N & Stamler J Experimental atherosclerosis Thomas Springfield 1953
57 Katz L N Stamler J & Pick R Nutrition and atherosclerosis Lea and Febiger Philadelphia 1958
58 Keys A J Mt Sinai Hosp 20 118–139 1953
59 — Nutr Rev 26 259–263 1968
60 — Circulation (Suppl) 1 1–211 1970
61 — Predicting coronary heart disease In Preventive cardiology (ed G Tibblin A Keys and L Werko) pp 21–31 Almqvist & Wiksell Stockholm 1972
62 — Mortality and coronary heart disease in the Mediterranean area In Proceedings of the II International Congress on the Biological Value of Olive Oil pp 281–286 Torremolinos Spain 1976
63 Keys A Anderson J T & Grande F Metabolism 14 747–787 1965
64 Keys A Aravanis C Blackburn H W et al Acta Med Scand (Suppl) 460 1966
65 Kleinbaum D G Kupper L L Cassel J C & Tyroler H A Arch Intern Med 128 943–948 1971
66 Knox E G Br J Prev Soc Med 31 71–80 1977
67 Kornitzer M De Backer G Dramaix M & Thilly C Intl J Epidemiol 8 23 1979
68 Kozarevic D Pirc B Racic Z Dawber T R Gordon T & Zukel W J Am J Epidemiol 104 133–140 1976
69 Kubler W Breithard D Gries F A et al Risk factors in West German patients with angiographically documented coronary heart disease In Atherosclerosis III (ed G Schettler and A Weizel) p 841 Springer Verlag New York 1974
70 Leren P Acta Med Scand (Suppl) 466 1966
71 Lopez S A Krehl W A Hodges R E & Good E I Am J Clin Nutr 19 361–369 1966
72 Mahley R W Bersot T P Innerarity T L Lipson A & Margolis S Lancet 2 807–809 1978
73 Malmros H Acta Med Scand (Suppl) 246 137–153 1950
74 Malmros H & Sternby N H Progr Biochem Pharmacol 4 482–487 1968
75 Marmot M G Syme S L Kagan A Kato H Cohen J B & Belsky J Am J Epidemiol 102 514–525 1975
76 Masironi F Bull WHO 42 103–114 1970
77 Mattson F H Erickson B A & Kligman A M Am J Clin Nutr 25 589–594 1972
78 McGee D & Gordon T The Framingham Study —an epidemiological investigation of cardiovascular disease Section 31 The results of the Framingham Study applied to four other U S based epidemiologic studies of cardiovascular disease U S Department of Health Education and Welfare DHEW Publication no (NIH) 76-1083 Washington D C 1976
79 McGill H C Jr (ed) Geographic pathology of atherosclerosis Williams & Wilkins Baltimore 1968
80 Medalie J H Kahn H A Neufeld H N Riss E & Goldbourt U J Chronic Dis 26 329–349 1973
81 Menotti A Capocaccia R Conti S et al J Chronic Dis 30 557–565 1977
82 Miettinen M Turpeinen O Karvonen M J Elosuo R & Paavilainen E Lancet 2 835–838 1972
83 Miller N E Førde O H Thelle D S & Mjøs O D Lancet 1 965–968 1977
84 Ministry of Agriculture Government of Norway Norwegian food and nutrition policy White paper no 32 1975–1976
85 Moriyama I M Krueger D E & Stamler J Cardiovascular diseases in the United States Harvard University Press Cambridge Mass 1971
86 Moriyama I M Woolsey T D & Stamler J J Chronic Dis 7 401–412 1958
87 Morris J N Marr J W & Clayton D G Br Med J 2 1307–1314 1977
88 Multiple Risk Factor Intervention Trial Research Group (Abstract) Circulation (Suppl) III 113 1977
89 National Advisory Council on Nutrition of the Netherlands Report Voeding 34 552–562 1973
90 National Diet Heart Study Research Group Circulation 37 (Suppl) 1 1968
91 National Heart Lung and Blood Institute Mortality trend data from the National Center for Health Statistics and the World Health Organization 1979
92 Nutrition Committee of the Steering Committee for Medical and Community Programs American Heart Association Diet and coronary heart disease American Heart Association 7-78-50M Dallas Texas 1978
93 Oliver M F Heady J A Morris J N et al Br Heart J 40 1069–1118 1978
94 Pahlke G Nutr Metab 18 (3) 113–115 1975
95 Paoletti R Gotto A (ed) Atherosclerosis reviews 1–4 Raven Press New York 1976 1977 1978 1979
96 Pelkonen R Nikkila E A Koskinen S Penttinen K & Sarna S Br Med J 2 1185–1187 1977
97 Phillips R L Lemon F R Beeson L & Kuzma J W Am J Clin Nutr 31 S191 1978
98 Pooling Project Research Group J Chronic Dis 31 201–306 1978
99 Pyorala K Savolainen E Lehtovirta E Punsar S & Siltanen E J Chronic Dis 32 729–746 1979

100 Reunanen A Pyorala K Aromaa A Maatela J & Knekt P J Chronic Dis 32 747–758 1979
101 Ringen K Am J Public Health 67 550–551 1977
102 Rinzler S H Bull NY Acad Med 44 936–949 1968
103 Robertson T L Kato H Gordon T et al Am J Cardiol 39 244–249 1977
104 Robertson T L Kato H Rhoads G G et al Am J Cardiol 39 239–243 1977
105 Rose G The basic principles of multifactorial prediction and control. Proceedings of a symposium From Epidemiology to Prevention pp 107–110 Megeve Switzerland 1974
106 Rose G Reid D D Hamilton P J S McCartney P Keen H & Jarrett R J Lancet 1 105–109 1977
107 Rosenman R H Brand R J Jenkins D Friedman M Straus R & Wurm M JAMA 233 872–877 1975
108 *Rosenman R H Brand R J Sholtz R I &* Friedman M Am J Cardiol 37 903–910 1976
109 Sacks F M Castelli W P Dorner A & Kass E H N Engl J Med 292 1148–1151 1975
110 Schettler G Prev Med 5 216–225 1976
111 — Prev Med 8 581–590 1979
112 Schettler G et al (ed ) Atherosclerosis III and IV Springer Verlag Berlin and New York 1974 1977
113 Schroll M & Hagerup L J Chron Dis 32 699–708 1979
114 Select Committee on Nutrition and Human Needs U S Senate Dietary goals for the United States U S Government Printing Office Washington D C 1977
115 Stamler J Lectures on preventive cardiology Grune & Stratton New York 1967
116 — Br Heart J 33 (Suppl) 145–164 1971
117 — Circulation 58 3–19 1978
118 — Improving life styles to control the coronary epidemic International Conference Nutrition Dietetics and Sports pp 5–48 Edizioni Minerva Medica Torino 1978
119 — Population studies In Nutrition lipids and coronary heart disease (ed R Levy B Rifkind B Dennis & N Ernst) pp 25–88 Raven Press New York 1979
120 — Improved life styles Their potential for the primary prevention of atherosclerosis and hypertension in childhood In Childhood prevention of atherosclerosis and hypertension (ed R Lauer and R B Shekelle) pp 3–36 Raven Press New York 1980
121 — The fat modified diet Its nature effectiveness and safety In Childhood prevention of atherosclerosis and hypertension (ed R Lauer and R B Shekelle) pp 387–403 Raven Press New York 1980
122 Stamler J Berkson D M & Lindberg H A Risk factors. Their role in the etiology and pathogenesis of the atherosclerotic diseases In Pathogenesis of atherosclerosis (ed R W Wissler and J C Geer) pp 41–119 Williams and Wilkins Baltimore 1972
123 Stamler J & Katz L N Circulation 2 705–713 1950
124 Stamler J Stamler R & Shekelle R B Regional differences in prevalence incidence and mortality from atherosclerotic heart disease In Ischaemic heart disease (ed J H de Haas H C Hemker and H A Snellen) pp 84–127 Leiden University Press Leiden 1970
125 Stamler R Stamler J Lindberg H A et al J Chronic Dis 32 805–816 1979
126 Stamler R Stamler J Schoenberger J A et al J Chronic Dis 32 817–828 1979
127 Stenhouse N S Murphy B P & Welborn T A J Chronic Dis 32 693–698 1979
128 St Leger A S Cochrane A L & Moore F Lancet 1 1017–1020 1979
129 Task Force on Arteriosclerosis of the National Heart and Lung Institute Arteriosclerosis vol 1 U S Department of Health Education and Welfare Public Health Service DHEW Publication no (NIH) 72–137 Washington D C June 1971
130 Tibblin G Wilhelmsen L & Werko L Am J Cardiol 35 514–522 1975
131 Tillotson J L Kato H Nichaman M Z et al Am J Clin Nutr 26 177–184 1973
132 Turpeinen O Circulation 59 1–7 1979
133 Turpeinen O Karvonen M J Pekkarinen M Miettinen M Elosuo R & Paavilainen E Intl J Epidemiol 8 99–118 1979
134 Tyroler H A Heyden S Bartel A et al Arch Intern Med 128 907–914 1971
135 Welch C C Proudfit W L & Sheldon W C Am J Cardiol 35 211–215 1975
136 White House Conference on Food Nutrition and Health Final Report U S Government Printing Office Washington D C 1970
137 White P D Sprague H B Stamler J et al A statement on arteriosclerosis main cause of heart attacks and strokes National Health Education Committee Inc New York 1959
138 Wilhelmsen L Wedel H & Tibblin G Circulation 48 950–958 1973
139 Wilhelmsson C Vedin J A Wilhelmsen L Tibblin G & Werkö L Lancet 2 1157–1160 1974
140 Winikoff B Am J Public Health 67 552 557 1977
141 Wissler R W Development of the atherosclerotic placque In The Myocardium Failure and Infarction (ed E Braunwald) pp 155–166 HP Publishing Co Inc New York 1974
142 Wissler R W & Geer J C (ed ) The pathogenesis of atherosclerosis Williams and Wilkins Baltimore 1972
143 Wissler R W & Vesselinovitch D Ann NY Acad Sci 275 363–378 1976
144 — Adv Vet Sci Comp Med 21 351–420 1977
145 Worth R M Kato H Rhoads G G Kagan A & Syme S L Am J Epidemiol 102 481–490 1975
146 Yerushalmy J & Hilleboe H E NY State J Med 57 2343–2354 1957
147 Yudkin J Lancet 2 155–162 1957

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# The Preleukemic Syndrome

## II Cytogenetic Findings

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ABSTRACT Out of 151 patients with preleukemic syndrome, bone marrow chromosome studies were carried out in 88 during the preleukemic phase and in 10 after blastic transformation Out of 54 cases studied without banding techniques, 13 (24%) were abnormal, while 17 (50%) out of 34 banded cases showed abnormalities This highly significant increase in yield of aberrations was not restricted to structural abnormalities During the preleukemic phase of the disease, only 5 of the abnormal patients had no normal metaphases in their bone marrow Four types of chromosome aberrations were observed more than once −Y +8, del 5q and del F or del 20q They are all frequently observed in myeloproliferative disorders After blastic transformation 15 out of 19 patients were abnormal and the abnormalities were more complex It seems, therefore, that a qualitative and quantitative difference exists between this group of patients and the published series of patients with ANLL Small abnormal cell lines with the same chromosome abnormalities as in the bone marrow were observed in PHA stimulated blood cultures of 9 patients Unstimulated cultures of the same blood sample did not show any mitosis It is suggested that small subpopulations of lymphocytes arose from the same pluripotent stem cell as the leukemic myelogenous cells, although there may be other explanations

*Key words* preleukemic syndrome chromosome abnormalities blastic transformation banding techniques pluripotential cells

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In a preceding article (24) we present the results of a long term follow up in patients with the preleukemic syndrome with special emphasis on clinical and hematomorphological investigations It is the purpose of this paper to present the cytogenetic findings in the same series of patients

The prospective nature of this study enabled us to relate the presence or absence of acquired chromosome abnormalities to the natural course of the disease and in particular to correlate the presence of clonal abnormalities with a more rapid evolution towards the onset of blastic transformation The chromosomes of a number of patients were studied also or exclusively after transformation to overt acute leukemia This approach enabled a quantitative and qualitative comparison of the observed aberrations with those seen in studies of patients with acute non lymphocytic leukemia (ANLL) The proportion of cytogenetic bone marrow abnormalities in patients with the latter diagnosis has been estimated to be about 50% and a number of changes seem to occur non randomly (1)

Since the study extended from the early 60s up to 1979 both unbanded and banded chromosome analyses were employed and the increased efficiency of chromosome banding techniques in this type of study could be estimated roughly

Also a more fundamental question evolved over the years whether the simultaneous presence of the same chromosome abnormalities in myeloid and lymphoid cells indicates the existence of pluripotential cells

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*Abbreviations* AML acute myelogenous leukemia ANLL=acute non lymphocytic leukemia CML=chronic myelogenous leukemia PHA=phytohemagglutini

Table I. Marrow chromosome studies in 88 patients during the preleukemic phase.
NN = normal metaphases only; AN = abnormal and normal metaphases; AA = abnormal metaphases only

| Group | Metaphases | Alive: With transformation | Alive: Without transformation | Dead: With transformation | Dead: Without transformation | Unknown | Total |
|---|---|---|---|---|---|---|---|
| Unbanded | NN | | 7 | 6 | 18 | 10 | 41 |
| | AN | | | 7 | 4 | 1 | [illegible] |
| | AA | | | | 1 | – | 1 |
| Banded | NN | 1 | 11 | [illegible] | 1 | 2 | 17 |
| | AN | | 7 | | 5 | 1 | 13 |
| | AA | 1 | 1 | [illegible] | – | | 4 |
| Total | | [illegible] | [illegible] | 17 | 29 | 14 | 88 |

## STUDY POPULATION

The criteria for inclusion of patients in this series are described in a preceding article (24), in which the clinical aspects and follow up studies are presented. Out of the potentially available 151 patients, 97 (45 males and 53 females) were studied cytogenetically, 88 of them during the preleukemic phase. In 33 cases no material was available for cytogenetic study, while in another 20 instances bone marrow metaphases were obtained in insufficient numbers for analysis.

## METHODS

All initial karyotyping was carried out on bone marrow chromosomes by the direct technique of Knlough et al. (7) with some modifications as described by Fitzgerald et al. (3), except in 2 cases where a 24-hour unstimulated peripheral blood culture was used. The same material was employed in 10 cases after blastic transformation. Peripheral blood was cultured for 72 hours with and without phytohemagglutinin (PHA) stimulation in a number of cases with a numerical chromosome abnormality in the bone marrow. This was done to detect the presence of lymphocytes with the same abnormal karyotype.

Air-dried slides were either stained conventionally with Giemsa or G-banded using a combined 2× sodium chloride trisodium citrate solution and trypsin pretreatment (5). Q-banding (17) and C-banding (22) techniques were employed occasionally. Chromosome abnormalities were designated using the recommendations of the Paris Conference supplement (1).

## RESULTS

At the time of the initial diagnosis of preleukemia 88 patients were studied chromosomally. According to the conventional staining or banding techniques used, they can be divided into two groups of 54 and 34 cases, respectively (Table I). The karyotypic abnormalities detected in these studies are presented in Tables II and III. Abnormalities were found in 13 (24%) of the patients in the unbanded group (Fig. 1) and in 17 (50%) in the banded group. A total of only 5 patients had no normal metaphases in their bone marrow. At the time of writing, 45 chromosomally investigated patients have died; in 17 of them the cause of death was related to a blast transformation. Since most of the patients studied with banding techniques were diagnosed more recently and are still alive, the correlation between presence of chromosome abnormalities and development of overt leukemia was analysed for only the deceased patients in the unbanded group. Patients with chromosome abnormalities had developed leukemia more frequently than those with normal karyotypes ($\chi^2=3.85$, $p<0.05$). However, the length of the follow up period did not differ between the two groups.

A total of 19 patients were studied after blastic transformation, 9 of whom were also examined during the preleukemic phase. The karyotypes of all metaphases are given in Tables III and IV. Only 4 of these patients had normal karyotypes after transformation.

Unstimulated blood cultures at the time of diagnosis revealed a sufficient number of metaphases in 4 patients, three of whom were chromosomally abnormal. PHA stimulated peripheral blood cultures were abnormal in 9 cases. In all of them except one the abnormal cell lines represented only a small proportion of the dividing cells. The exception (patient 68) was a male who was mosaic for the Kl ne

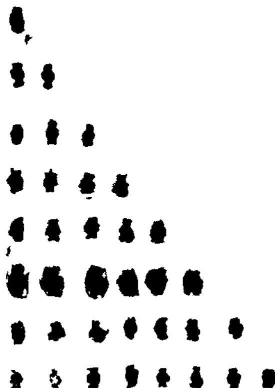


*Fig. 1* Selection of dicentric chromosomes from bone marrow cells of patient 96 Conventional staining

felter syndrome his buccal smear was X chromatin positive and histological examination of a testis biopsy confirmed the diagnosis

## DISCUSSION

In the largest published series of patients with suspected preleukemia to date (13) 205 direct bone marrow chromosome studies resulted in a 36% abnormality rate This figure lies between our figures of 24% for unbanded and 50% for banded analyses The observed increase in yield of aberrations following the application of banding techniques is statistically significant ($\chi^2 = 6.24$ d f = 1 $p<0.02$) If the aberrations are divided into numerical structural and the combination of numerical and structural all three groups are observed more frequently among the banded patients The increased yield of aneuploidies is somewhat surprising After the observation of three cells with the same missing or extra chromosome the application of banding techniques permits the conclusion that an aneuploid cell line is present Our conclusion is therefore that although less cells are available for banding analysis it is in general easier to detect a chromosome abnormality with than without banding providing the banding procedure works reproducibly well

Due to the length of the study period small changes in diagnostic criteria or etiology of the preleukemic syndrome might partly explain the observed difference in yield of aberrations between unbanded (earlier) and banded (later) material In the unbanded group 36 patients have died 13 of them after transformation and 7 of these 13 were chromosomally abnormal This means that leukemia was more frequent among the patients with than without chromosome abnormalities Most patients in the banded group are still alive and the number of dead patients until now is too small to ascertain whether the same phenomenon is operating here too Most of the patients were 70–80 years old and death due to other causes than acute leukemia is likely to occur in a significant proportion

In all but four cases studied in the preleukemic phase the chromosome abnormality was clonal and present together with karyotypic normal cells Therefore they can be classified as AN (abnormal and normal) according to Sakurai and Sandberg (21) This type of abnormality has been claimed to carry a better prognosis than the AA (abnormal) type when observed in patients with acute myelogenous leukemia (AML) (6) Four types of chromosome aberrations were observed more than once namely −Y +8 del 5q and del F or del 20q They are all frequently observed in myeloproliferative disorders The loss of a Y chromosome occurs also in normal males at advanced ages (9) Although most of our patients are within this category the finding of 7 cases with Y chromosome aneuploidy might be related to the presence of a bone marrow abnormality Arguments for such an association stem from the following facts A missing Y chromosome has been found in about 10% of males with t(9 22)-positive chronic myeloid leukemia (21) Curiously enough also t(8 21) which is associated with acute myeloid leukemia is frequently associated with loss of the Y (16 21) This type of aneuploidy has been documented before in preleukemia (14 15 19) Y-chromosome loss was found recently in bone marrow cells of two chil-

Table II *Patients with an abnormal karyotype in the preleukemic phase*
B = banded U = unbanded

| Case no | Age (y) | Sex | Bone marrow | Unstimulated blood | Stimulated blood |
|---|---|---|---|---|---|
| 5 B | 75 | ♀ | 46,XX=20<br>47,XX +8=11<br>47,XX +8 del(21)(q21)=4 | No metaphases | 46,XX=50<br>47,XX +8=8<br>47,XX +8 del(21)(q21)=3 |
| 8 U | 75 | ♀ | 46,XX=18<br>48,XX +C +C=13 | 46,XX=20<br>48,XX +C +C=6 | |
| 10 U | 85 | ♂ | 46,XY=25<br>46,XY del(F)=10 | | |
| 24 U | 83 | ♂ | 46,XY=11<br>47,XY +C=24<br>48,XY +C +C=5 | No metaphases | 46,XY=255<br>47,XY +C=10 |
| 28 B | 71 | ♂ | 46,XY=8<br>45,X −Y=4 | | |
| 29 B | 73 | ♂ | 46,XY=16<br>45,X −Y=4 | | |
| 32 B | 81 | ♂ | 46,XY=59<br>46,XY t(2;3)(q?;p?)=1 | | |
| 37 B | 53 | ♂ | 46,XY=86<br>47,XY +8=13<br>47,XY +21=1 | | |
| 41 U* | 77 | ♂ | 46,XY=1<br>47,XY +F=18 | No metaphases | 46,XY=241<br>47,XY +F=25 |
| 43 B | 78 | ♀ | 46,XX=15<br>46,XX *del(5)(q13q31)*=7 | | |
| 58 B | 60 | ♂ | 46,X −Y +t(1;?)(p?;?)=20 | | |
| 68 U | 69 | ♂ | 46,XY=64<br>47,XY +C=258<br>48,XY +C +C=48<br>49,XY +C +C +G=12<br>50,XY +C +C +G +G=1 | No metaphases | 46,XY=133<br>47,XY +C=153<br>48,XY +C +C=45<br>49,XY +C +C +G=10<br>50,XY +C +C +G +G=2 |
| 70 U | 57 | ♀ | 46,XX=4<br>47,XX +C=3<br>45,XX −G=2 | 46,XX=25<br>47,XX +C=5<br>45,XX −G=4 | |
| 87 B | 54 | ♀ | 46,XX *del(5)(q13q23)*=10<br>47,XX +21 del(5)(q13q23)=1 | | |
| 88 B | 6 | ♀ | | 46,XX=1<br>47,XX +6=5<br>47,XX +6 t(18;?)(q23;?)=4 | |
| 90 B | 60 | | 46,XY=1<br>46,X −Y +8=19 | No metaphases | 46,XY=18<br>46,X −Y +8=11<br>47,XY +8=3 |
| 96 U* | 61 | | 46,XY=13<br>47,XY +mar1=4<br>48,XY +2mar1=16<br>49,XY +3mar1=29<br>50,XY +4mar=53<br>51,XY +5mar=11<br>52,XY +6mar=6<br>53,XY +7mar=8<br>54,XY +8mar=1 | No metaphases | 46,XY=1330<br>47,XY +mar1=30<br>48,XY +2mar1=4<br>49,XY +3mar1=2 |
| 97 B | [illegible]0 | ♂ | 46,XY=[illegible]0<br>47,XY +8=13 | No metaphases | 46,XY=90<br>47,XY +8=23<br>47,XY +Y=1 |
| 103 U* | 66 | ♀ | 46,XX=41<br>46,XX del(G)(q?)=5 | Not done | 46,XX=47<br>46,XX del(G)(q?)=29 |

Table II (cont )

| Case no | Age (y) | Sex | Bone marrow | Unstimulated blood | Stimulated blood |
|---|---|---|---|---|---|
| | | | 45 XX −C del(G)(q?)−45<br>47 XX +del(G)(q?)=27 | | 45 XX −C del(G)(q?) 10<br>47 XX +del(G)(q?)=12 |
| 104 U | 84 | ♀ | 46 XX=3<br>46 XX del(F)=15 | | |
| 113 B | 45 | ♀ | 46 XX 9<br>47 XX +8=2 | No metaphases | 46 XX=182<br>47 XX +8 8 |
| 121 U* | 59 | ♂ | 46 XY=34<br>47 XY +C=4 | | |
| 125 B | 75 | ♂ | 46 XY 17<br>45 X −Y=5 | | |
| 127 U* | 57 | ♀ | 46 XX−6<br>47 XX +C 4 | | |
| 130 U | 79 | ♀ | 49 XX +D +D +F=2<br>50 XX +C +D +D +F=2 | | |
| 144 B | 76 | ♂ | 46 XY=4<br>45 X −Y=16 | | |
| 149 B | 81 | ♂ | 46 XY=25<br>46 XY del(7)(q11q22)=25 | | |

Developed leukemia later

dren one with acute myeloblastic leukemia and t(8 21) and the other with acute lymphoblastic leukemia (10) Especially the latter findings suggest a role of the Y chromosome loss in leukemogenesis Five of our patients one of them after blastic transformation (nos 28 29 60 125 and 144) had the missing Y as the only abnormality while patient 58 had an extra re arranged chromosome 1 and patient 90 had an extra copy of chromosome 8

Trisomy 8 is most probably the most common single chromosome abnormality in our material since we can assume that apart from the five banded cases with this abnormality a number of extra C group chromosomes (in altogether 10 patients) will




*Fig 2* Karyotype of bone marrow cell from patient 43 Giemsa banding Note deletion of long arm of chromosome 5

Table III *Patients studied before and after blastic transformation*

B= banded U= unbanded

| | | | Before transformation | | | | After transformation | | |
|---|---|---|---|---|---|---|---|---|---|
| Case no | Age (y) | Sex | Tissue | Patient group | Karyotype | Age + months | Tissue | Patient group | Karyotype |
| 19 | 67 | ♂ | Marrow | U | 46 XY=25 | 39 | Marrow | U | 46 XY=35 |
| | | | | | | | | | 52 XY +B +B +C +C +C +D=5 |
| 36 | 81 | ♀ | Marrow | U | 46,XX=30 | 54 | Blood−PHA | B | 46 XX=5 |
| 83 | 67 | ♀ | Marrow | B | 46 XX 1p+ 9q− 18q+ r(20)=16 | 6 | Marrow | B | 46 XX 1p+ 9q− 18q− r(20)=17 |
| 102 | 76 | ♀ | Marrow | U | 46 XX=10 | 8 | *Blood−PHA* | *B* | 46 XX−8 |
| | | | | | | | | | 46,XX del(5) del(6) del(7) 21q+=7 |
| 109 | 92 | ♂ | Marrow | B | 46 XY=10 | 8 | Marrow | B | 46 XY=25 |
| 123 | 81 | ♀ | Marrow | U | 46,XX=20 | 36 | Blood−PHA | B | 46,XX=15 |
| 138 | 56 | ♀ | Blood−PHA | B | 46,XX=22 | 3 | Blood−PHA | B | 46 XX t(3 11) (q12 p13)=10 |
| 67 | 57 | ♀ | Marrow | U | 48,XX +C +C−29 | 67 | Marrow | B | 46 XX +8 +9=28 |
| | | | Blood + PHA | U | 46 XX=829 | | Blood + PHA | B | 46 XX=1158 |
| | | | | | 47 XX +C=84 | | | | 47 XX +8=3 |
| | | | | | 48 XX +C +C=27 | | | | 48 XX +8 +9−33 |
| | | | Blood − PHA | U | 48 XX +C +C=10 | | Blood − PHA | B | 48,XX +8 +9=85 |
| 145 | 79 | ♀ | Marrow | B | 43 XO −3 −13 4q− 8q− 12q+=29 | | | | |
| | | | Blood − PHA | | No metaphases | | | | |
| | | | Blood + PHA | B | 46 XX=10 | 6 | Blood PHA | B | 46 XX=20 |
| | | | | | 43 WO 3 −3 4q− 8q 12q+=7 | | | | 43 XO −3 −13 4q− 8q− 12q+=14 |

represent this chromosome An extra chromosome 8 is the most common abnormality in ANLL as well as the most frequent change in t(9 22)-positive chronic myeloid leukemia in the acute phase (18) A hint towards the leukemogenecity of this abnormality comes from the observation of Gafter et al (4) whose patient with congenital trisomy 8 mosaicism suffered initially from aplastic anemia which was followed by leukemia The presence of this abnormality does not necessarily mean that the prognosis is very much altered Our patient 97 for example was first seen in 1966 at the age of 40 At that time an extra C group chromosome was present in the bone marrow and peripheral blood lymphocytes but not in skin fibroblasts He died in 1979 of an unrelated cause without signs of blastic transformation Follow up of the cytogenetic examination disclosed that the extra chromosome was no 8

A deletion of the long arm of chromosome 5 was observed in 4 cases (Fig 2) This 5q abnormality has been reported before in various myeloproliferative disorders mainly refractory anemias but also in AML (8) This abnormality was found after blastic transformation in two of our patients (nos 102 and 110) The former patient was studied also during the preleukemic phase and was normal at that time After development of overt leukemia *deletions of chromosomes 6 and 7 were also noted* In this respect our patient resembles one of Rowley s patients who had deletions of all three chromosomes among other abnormalities and was diagnosed as having erythroleukemia (17)

Finally a deletion of an F group chromosome was observed more than once in our series It is known that deletions of the long arm of chromosome 20 occur quite frequently in myeloproliferative disorders especially polycythemia vera but the same aberration has also been found in a patient who possibly had preleukemia (23) Our conclusion is therefore that about half of our patients had chromosome abnormalities during the preleukemic phase and that most of these abnormalities have also been found in patients with various other myeloproliferative disorders At this stage we are unable to connect specific clinical findings to any of the above mentioned specific chromosome aberrations

The frequency of abnormalities of almost 60%

Table IV *Preleukemic patients studied after blastic transformation only*

B banded U unbanded

| Case no | Age (y) | Sex | Bone marrow | Unstimulated blood | Stimulated blood |
|---|---|---|---|---|---|
| 16 U | 58 | ♀ | 46,XX=69<br>47,XX,+C=49 | | 46,XX=169<br>47,XX,+C=23 |
| 26 U | 78 | ♀ | 46,XX=212<br>47,XX,+C=2<br>48,XX,+C,+C=5<br>49,XX,+C,+C,+D=25 | No metaphases | 46,XX=187<br>47,XX,+C=6<br>47,XX,+C,del(F)=7<br>48,XX,+C,+C=2 |
| 34 B | 79 | ♂ | 46,XY=22<br>46,XY,t(3?)(q26?)=8 | | |
| 47 U | 83 | ♂ | Chromosome breakage | | Chromosome breakage |
| 60 B | 90 | ♂ | 45,X,−Y=50 | | 45,X,−Y=50 |
| 62 B | 77 | ♂ | 46,XY=1<br>45,XY,−9,13q−=1<br>45,XY,−9,13q−,5q−=1 | 2n=6<br>(highly abnormal) | |
| 72 B | 83 | ♀ | | 46,XX=48<br>47,XX,+10,del(1)(p22)=54 | |
| 73 B | 72 | ♀ | 47,XX,+t(6?)(q12?)=20 | | |
| 110 B | 80 | ♂ | | 46,XY,del(5)(q13q31)=20 | |
| 139 U | 83 | ♀ | 46,XX=20 | | |

after blastic transformation (15 of 19 patients) is higher than that of 50% observed in a large series of ANLL (1). These abnormalities had no consistent pattern. Structural re-arrangements of different types (deletions, translocations) and different chromosomes were observed in 9 patients. It is therefore possible that a qualitative and quantitative difference exists between this group of our patients and the ANLL patients mentioned above.

The last problem is related to the observation in patients 5, 24, 41, 68, 90, 96, 97, 103, 113 of small PHA responsive cell clones with the same chromosome abnormalities as in the bone marrow. In these patients, an unstimulated culture from the same blood sample did not show any mitosis. The most obvious explanation of these findings seems to be the presence of a small subpopulation of lymphocytes which arose from the same pluripotent stem cell as the leukemic myelogenous cells. Evidence for the existence of such subpopulations has recently been demonstrated by enzyme studies in three female patients with chronic myelogenous leukemia (CML) (2). These were heterozygotes for the X-linked marker glucose-6-phosphate dehydrogenase and by E rosetting it was possible to isolate cells with T lymphocyte characteristics displaying the single enzyme phenotype that was present in the myeloid cells. This population was demonstrated when the disease was under poor control. Although only a few mitoses obtained after PHA stimulation of these lymphocytes were Ph chromosome positive, it was considered possible that the cells in metaphase were not representative of the vast majority of enzyme producing cells. Indirect evidence for the involvement of a lymphocyte series in CML is obtained from the observation that some patients have cells with lymphoblastic features in their peripheral blood after blast cell transformation. Other explanations of the presence of chromosomally abnormal cells in the PHA stimulated cultures are however possible. The possibility of a direct stimulation by the PHA of circulating stem cells themselves cannot be ruled out. Another possibility is the release of substances in the culture medium by the stimulated lymphocytes which promote mitotic activities of myeloid progenitor cells.

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## REFERLNCES

1 Van den Berghe H Bo g t om G H B andt L et al F st In ernat on d Wo kshop on Ch on osomes n leukem a Cancer Re 38 867 19 8
F alkow J J D nman A M Ja ob on R J & Lowen hal M N Chron myelocyt c leukem a Orig n of some lymph f om leukem c stem ce ls J Cl n In e t 6 81 1 8
3 F tzgerald P H A lam A & Gu z F W Chron c granulo yt c l ken a t he I h delph a ch omosome Fl od 8 96
4 Gal er t Sh b a F Kahn Y Halbrecht I & Dja de 1 Apl c naem a t wed by leukem a n n omy 8 n o c m Cl n Genet 9 134
5
I M & Haak H L Trisomy 6 as a aem a Hum Genet 35 113

I M d man J W Rowley J D
T & M n z U Correlat on of cl n cal f nd qu nne banded chromosomes n 90 acut non lymphocy c leukem a N Engl J 6 3 1978
h M A Robson H N & Hayman D L I d me hod for he study of chromosomes in Na ur 189 4 0 1961
1 n n F & Le an G Clustering of aberrat ons pe fc h omosomes n human neoplasms III In den e and geograph c d str but on of chromosome be a on n 856 cases Hered tas 89 07 1978
O R ordan M L Berry E W & Tough I M Cl romosome stud es on bone marrow from a male control populat on Br J Haematol 19 83 1970
10 Pad e Mendoza T Farnes P Barker B E Sm th P S & Forman E N Y ch omosome loss n ch ld hood leukaem as Br J Haematol 41 43 1979
11 Paris Conference (1971) (Suppl) Standardization in human cytogenet cs B rth defects Or g nal art cle se es vol XIV no 8 The Nat onal Foundat on New Yo k 1978
12 Pearson P L Bobrow M & Vosa C Techn que for identify ng Y chromosomes in I interphase nuclei Nature 2 6 78 1970
13 Pierre R V Preleukem c states Sem n He 11 73 1974
14 Pierre R V & Hoagland H C Age assoc aneuplo dy loss of Y chromosome from human I marrow cells w th ag ng Cancer 30 889 1972
15 Rowley J D Loss of the Y chromosome n my dy plas a a report of three cases stud ed w th qu t rine fluorescence Br J Haematol 31 717 1974
16 — M ss ng sex ch omosomes and translocat ons acute leukem a (letter) Lancet 2 835 1974
17 — Populat on cytogenet cs of leukem a In P pu t on cytogenet cs (ed E B Hook and I H Porte ) 169 Academ c Press New York 1977
18 — Chromosomes in leukem a and lymphoma Sen Hematol 15 301 1978
19 Sakura M Chromosome stud es n haematolog disorders III Chromosome f nd ngs n prel kem a and related d seases Acta Haematol Jpn 177 1970
20 Sakurai M & Sandberg A A Prognos s of ac myeloblast c leukem a chromosomal correlat Blood 41 93 1973
21 — The chromosomes and causations of hum cancer and leukem a XVIII The m ss ng Y n ac myelobast c leukem a (AML) and Ph pos t chron c myelocyt c leukem a (CML) Cancer 38 7 1976
22 Sumner A T A simple techn que for demonstrat centromer c heterochromat n Exp Cell Res 75 3 1972
23 Testa J R Kinealy A Rowley J D Golde W & Potter D Delet on of the long arm of chrom some 20 (del ( 0) (q11)) n myelo d d sorders Bl 52 868 1978
24 Weber R F A Geraedts J P M Kerkhofs H Leeksn a C H W The preleukem c syndrome Cl n cal and haematolog cal f nd ngs Acta Med Sc 207 391 1980

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# Hemoperfusion with Amberlite Resin in the Treatment of Self-Poisoning

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ABSTRACT Ten patients with various intoxications were treated with resin hemoperfusion Three of four patients with grade IV coma due to tricyclic antidepressant (TCA) poisoning could be extubated during or on termination of hemoperfusion Clearance values of 135–185 and 190–200 ml/min were obtained for amitriptyline and nortriptyline respectively One patient with severe chloral hydrate poisoning could be extubated after less than one hour s hemoperfusion A clearance of 140 ml/min was obtained in a uremic patient with AV block II due to digitoxin intoxication Four patients with mushroom poisoning were treated with combined hemoperfusion–hemodialysis A transient fall in platelet count was seen in all patients Resin hemoperfusion is of definite value in selected severe cases of self poisoning with psychotropic drugs such as TCA and possibly in cases of mushroom poisoning where the prognosis with hemodialysis and supportive therapy is doubtful

*Key words* intoxication hemoperfusion resin
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Self poisoning is a common cause of admission to hospital especially in large cities Many of these patients require intensive care In a select group of severe cases it may be advantageous to remove the drug as quickly as possible for example if drug induced cardiac arrhythmias are life threatening if there is a risk of permanent kidney or brain damage or if prolonged coma indicates tracheostomy

Forced diuresis peritoneal dialysis and hemodialysis are of limited help in most instances of self poisoning with psychotropic drugs such as tricyclic antidepressants (TCA) (2 13 17) Hemoperfusion with a charcoal filter first introduced in 1964 (22) is more effective than hemodialysis and has been proven effective in several poisonings including barbiturate glutethimide methaqualone salicylate paracetamol (8) and digitalis poisoning (4 18) but reports differ as to its value in TCA poisoning (3 7)

The Amberlite XAD-4 non ionic resin was first introduced by Rosenbaum et al (14) in 1970 as an alternative to the charcoal filter It has been proven effective in a number of cases of self poisoning but has been little used in TCA or mushroom poisoning

## METHODS AND MATERIAL

The hemoperfusion system consisted of a 650 g Amberlite® resin column in a Lexan cartridge (Extracorporeal USA)

Standard blood tubings (Gambro Sweden) employed in hemodialysis were used to connect the components in the circuit The treatment was controlled with a blood monitor device (Gambro) for measurement of blood pressures on the inflow and outflow sides of the cartridge blood flow and inhibition of air embolization by an air detector (Fig 1)

The system was perfused with 3–4 l heparinized isotonic saline 2 500 IU heparin/l The priming volume for the system is approximately 550 ml During perfusion parenteral heparin was given continuously at a rate of 1 500–2 000 IU/hour

Both femoral veins were catheterized percutaneously using a Cobe catheter with a tapered end and an inner diameter of 0 80 mm One catheter was used as the inflow (arterial) line to the cartridge and the other as the outflow (venous) line In two patients however Scribner shunts were used for access to blood After catheterization 5 000 IU heparin were given Perfusion was started about 10 min later at a flow of 150–300 ml/min and continued until a satisfactory clinical response had been obtained or up to 4 hours

Before starting and after perfusion hemoglobin hematocrit and platelet counts were estimated as well as serum electrolyte total protein calcium glucose creatinine bilirubin alkaline phosphatase aspartate transaminase and lactate dehydrogenase concentrations D

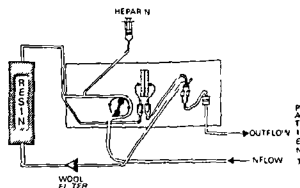



*Fig 1* The hemoperfusion circuit

ing hemoperfusion hemoglobin platelet count and activated partial thromboplastin time were measured frequently Plasma concentrations of TCA were analyzed before during and after perfusion by a gas chromatographic method (12) in the TCA intoxicated patients From two of the four TCA cases samples were taken on each side of the cartridge so that clearance could be calculated Plasma concentrations of digitoxin (case 6) were measured using a radioactive immunoassay technique (Diagnostic Products Corporation USA)

*Criteria for hemoperfusion in cases of drug induced* self poisoning were either respiratory depression or severe arrhythmias such as multifocal ventricular extrasystole or ventricular fibrillation Gastric aspiration was carried out in all patients irrespective of the time of ingestion after ensuring a patent airway When necessary assisted ventilation was given as well as sodium bicarbonate if acidosis was suspected

Hemoperfusion was combined with hemodialysis in four cases of mushroom poisoning where the mushroom taken was suspected of being highly toxic In two of these cases Cortinarius speciosissimus had been identified before admission to this hospital

A neurological assessment was made of the patient s level of consciousness according to Arieff and Friedman (1) reflex activity and pupil response both before and during hemoperfusion Likewise the pulse rate blood pressure respiration rate and response to voice and pain were registered

Ten patients (7 males and 3 females) with a mean age of 37 years (range 23–47) received a total of 14 hemoperfusion treatments

## RESULTS

*Ingestion of TCA was the reason for hemoperfusion* in 4 patients chloral hydrate and digitoxin in one patient each and ingestion of mushroom in 4 patients

The clinical response of each patient is summarized in Table I together with relevant changes in blood chemistry Three TCA patients were extubated during or on termination of hemoperfusion One patient (no 5) with chlorimipramine poisoning who was hemoperfused for only one hour could be extubated after a further 12 hours

Plasma drug concentrations of amitriptyline nortriptyline (a metabolite of amitriptyline) and digitoxin are given in Table II Clearance at a blood flow rate of 200 ml/min was 135–185 ml/min for amitriptyline and 190–200 ml/min for nortriptyline Extraction ratios were 0 65–0 93 for amitriptyline and 0 95–1 00 for nortriptyline

A patient with severe chloral hydrate intoxication required repeated cardioversion for VF and could be extubated after 50 min of hemoperfusion This patient had grade IV coma at the start of hemoperfusion and was awake four hours later when the hemoperfusion was terminated

A patient with uremia (no 6) was scheduled for a kidney transplant but showed an AV block II and had a PQ interval of 0 30 sec prior to operation caused by an overdose of digitoxin He was treated with combined hemoperfusion–hemodialysis Digitoxin clearance was 140 ml/min at a blood flow rate of 240 ml/min This patient had a normal ECG with a PQ time of 0 20 sec on the day after dialysis–perfusion and received a kidney transplant as planned In this case we had problems with coagulation in the hemoperfusion filter and the hemoperfusion was discontinued after 35 min This may explain why there was only a small reduction of the initial digitoxin concentration from 0 024 μmol/l before treatment to 0 020 μmol/l on the day after hemodialysis–hemoperfusion

In 4 of the 14 hemoperfusions variations in blood flow during perfusion and coagulation in the filter ruled out a reliable estimate of the amount of drug removed

Four patients were treated for mushroom poisoning At follow up three have normal serum creatinine values while the final prognosis concerning kidney function of the fourth patient is still uncertain After mushroom ingestion one patient was first treated with hemoperfusion and on the following day with hemodialysis because of clinically suspected ingestion of Amanita phalloides analysis of the stomach content revealed later that this patient had eaten the non toxic Macrolepiota rhacodes Two patients each who had ingested Cortinarius speciosissimus received three hemoperfusions and hemodialyses in which the perfusion cartridge was serially coupled to a dialyser (area 1 0

Table I *Details of patients and response to treatment*

| Pat no | *Age (y) | Sex | Poisoning | Condition | Duration of perfusion | Interval between start of perfusion and extubation |
|---|---|---|---|---|---|---|
| 1 | 44 | ♂ | Chloral hydrate | Grade I\ coma repeated ventricular fibrillation | 3 h -0 min | \0 min |
| 2 | 34 | ♂ | Amitriptyline | Grade IV coma | 1 h ,0 min | 1 h |
| 3 | 23 | ♀ | Amitriptyline | Grade IV coma | 35 min | 1 h |
| 4 | 31 | ♂ | Trimipramine | Grade IV coma sinus tachycardia | 4 h 13 min | 4 h 18 min |
| 5 | 33 | ♂ | Chlorimipramine propiomazine | Grade IV coma | 60 min | 12 h |
| 6 | 44 | ♂ | Digitoxin | Uremia AV block II awake | 35 min | – |
| 7 | 24 | ♂ | Cortinarius speciosissimus | Nausea single vomiting abdominal pain awake | 4 h + 4 h +4 h | |
| 8 | 47 | ♀ | Cortinarius speciosissimus | Nausea abdominal pain awake | 4 h + 4 h +4 h | – |
| 9 | 44 | ♀ | Macrolepiota rhacodes | Diarrhea awake | 40 min[b] | – |
| 10 | 41 | ♂ | Unspecified mushroom | Awake | 3 h | |

* Combined with hemodialysis on each occasion
[b] Hemodialysis on the following day

m ) One patient with acute anuria clinically suggesting intoxication with Cortinarius species was also treated with a serially coupled perfusion cartridge and a dialyser There were no significant changes in the serum concentrations of electrolytes calcium glucose bilirubin protein alkaline phosphatase ALAT or ASAT before and efter hemoperfusion in any patient No hemodynamic changes occurred during hemoperfusion A moderate fall in total platelet count was seen in all patients as well as a fall in hemoglobin concentration The fall in platelet count was pronounced in cases with clotting complications in the filter One patient developed a minor hemorrhage at the site of catheterization in the left groin This complication was related to heparin administration and was controlled by protamin All patients made a full recovery Four of the 14 hemoperfusion treatments given were discontinued because of coagulation in the filter

## DISCUSSION

Since 1964 both charcoal and resin columns have been shown to be effective in the treatment of intoxications with many drugs including barbiturates glutethimide paracetamol methaqualone salicylate and diazepam (5 18) Trafford et al (20) used resin hemoperfusion in one case each of chlorimipramine and amitriptyline poisonin. Their clearance rates for amitriptyline and nortriptyline agree with the present data (Table III) and with those recently published by others (3) Our extraction ratios for amitriptyline (0 65–0 93) agree with that found by Topf et al (19) who also used a resin

Table II *Plasma drug concentrations clearance values and extraction ratios*

| Pat no | Drug | Max plasma conc (μmol/l) | Inflow conc (μmol/l) | Outflow conc (μmol/l) | Blood flow (ml/min) | Clearance (ml/min) | Extraction ratio |
|---|---|---|---|---|---|---|---|
| 2 | Amitriptyline | 4 4 | 2 3 | 0 8 | 200 | 135 | 0 65 |
| | Nortriptyline | | 0 4 | 0 0 | 200 | 200 | 1 00 |
| [illegible] | Amitriptyline | 4 24 | 2 29 | 0 16 | 200 | 185 | 0 93 |
| | Nortriptyline | – | 2 37 | 0 11 | 200 | 190 | 0 95 |
| 6 | Digitoxin | 0 0.4 | 0 018 | 0 0075 | 240 | 140 | 0 58 |

Together with hemodialysis

13 Oreopoulos D G & Lal S Recovery from massive amitriptyline overdosage Lancet 2 221 1968
14 Rosenbaum J Winsten S Kramer M S Moros J & Raja R Resin hemoperfusion in the treatment of drug intoxication Trans Am Soc Artif Intern Organs 16 134 1970
15 Smith T W Haber E & Yeatman L Reversal of advanced digoxin intoxication with fragments of digoxin specific antibodies N Engl J Med 294 797 1976
16 Stalker N Gambertoglio J Fukumitsu C Naughton J & Benet L Acute massive chloral hydrate intoxication treated with hemodialysis A clinical pharmacokinetic analysis J Clin Pharmacol 18 136 1978
17 Steel C M O Duffy J & Brown S S Clinical effects and treatment of imipramide and amitriptyline poisoning in children Br Med J 3 663 1976
18 Tobin M & Mookerjee B Charcoal hemoperfusion in digitalis intoxication Dialysis and Transplantation 7 614 1978
19 Topf G Schultz W & Bartels O MAD-4-Hamoperfusion bei Amitriptyline Intoxikation Proceedings of Arbeitstagung uber Hamoperfusion 9 6 1978 p 73 Braun Fraba 1978
20 Trafford J Jones R Evans R Sharp P Sharpstone P & Cook J Hemoperfusion with R 004 Amberlite resin for treating acute poisoning Br Med J 2 1453 1977
21 Wauters J Rossel C & Farquet J Amanita phalloides poisoning treated by early charcoal hemoperfusion Br Med J 2 1465 1978
22 Yatzidis H A convenient hemoperfusion microapparatus over charcoal for the treatment of endogenous and exogenous intoxications Its use as an artificial kidney Proc Eur Dial Transplant Assoc 1 [illegible]

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# A Modified $^{125}$I-Fibrinogen Technique in Suspected Deep Vein Thrombosis

## *A Comparison with Plethysmography and Phlebography*

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ABSTRACT The diagnostic efficiencies of a modified $^{125}$I-fibrinogen uptake test (FUT), venous strain gauge plethysmography and the routine report on phlebography were compared in 301 consecutive patients with suspected deep vein thrombosis (DVT) in the leg A keen, independent review of the phlebography films was used as the reference method The FUT detected 62% of all thrombi after one hour 71% after one day, and 98% after two days False positive results were however, found after two days in 52% of all patients without DVT Plethysmography revealed 63% of the thrombi and was falsely positive in 23% of patients without DVT The routine examination of phlebography films revealed only 86% of the thrombi seen at the final independent review The routine report was falsely positive in 6% of patients without thrombi Consequently, the modified FUT is a useful screening test, at one hour it was equally sensitive to DVT as plethysmography and after two days it excluded DVT with a significantly better sensitivity than the routine report on phlebography Drawbacks of FUT are the delay of the diagnosis in some patients and the low specificity In patients with a pathological FUT, further investigation is often required Plethysmography is not recommended as a screening test, since it lacks both sensitivity and specificity

*Key words* deep vein thrombosis diagnosis $^{125}$I fibrinogen uptake test phlebography plethysmography

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It is generally agreed that clinical signs and symptoms are not reliable for the diagnosis of deep vein thrombosis (DVT) (10 16 22) Therefore many workers have tried to develop simple screening tests Hallbook and Gothlin (11) found venous strain gauge plethysmography to be useful but in routine work it proved to be less reliable (13) To obtain an alternative screening test in suspected DVT the $^{125}$I fibrinogen uptake test (FUT) was modified (17)

The aim of the present study was to compare the diagnostic efficiency of the modified FUT with that of strain gauge plethysmography and with the result of the initial examination of the phlebography films An independent careful review of the films was made later on the result of this review was regarded as the final reference However during the study it was found that phlebography often induced a pathological FUT and that this was often due to thrombosis (1 3) Therefore a second series of patients was studied in which phlebography was performed with a low osmolar contrast medium that did not cause thrombosis (2 3) In the second series plethysmography was replaced by a new diagnostic test using $^{99}$Tc$^{m}$ labelled plasmin (6) The results of the plasmin test will be reported separately (18)

## PATIENTS AND METHODS

A total of 301 patients were included in two series of consecutive patients (Nov 10 1975–April 15 1976 and March 14–May 29 1977) The first series (CS I) comprised 195 patients and the second (CS II) 106 patients (Table I) All patients seeking medical care at the emergency outpatient service with even a minor suspicion of DVT were included during these two periods On admission each patient was informed on the aims of our study

The tests were evaluated according to their sensitivity and specificity Sensitivity was defined as the proportion

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*Abbreviations* DVT = deep vein thrombosis FUT = $^{125}$I fibrinogen uptake test TP = true positive TN = true negative FP = false positive FN = false negative CS I = first patient series CS II = second patient series

Table III *Per cent of correctly diagnosed legs according to three different criteria in the modified FUT*

Results from CS I are included only if they were obtained before phlebography

| Findings at an independent review of phlebographies | Legs (*n*) | Criteria | | |
|---|---|---|---|---|
| | | 5-5-5% | 5-4-4% | 4-4-4% |
| *One hour after injection* | | | | |
| Thrombosis | | | | |
| All | 108 | 53 | 62 | 62 |
| Proximally | 62 | 74 | 82 | 82 |
| Distally | 46 | 24 | 35 | 35 |
| Not thrombosis | 145 | 78 | 72 | 72 |
| *After one day* | | | | |
| Thrombosis | | | | |
| All | 52 | 65 | 71 | 71 |
| Proximally | 21 | 95 | 100 | 100 |
| Distally | 31 | 45 | 52 | 52 |
| Not thrombosis | 63 | 81 | 73 | 73 |
| *After two days* | | | | |
| Thrombosis | | | | |
| All | 42 | 90 | 98 | 98 |
| Proximally | 26 | 96 | 100 | 100 |
| Distally | 16 | 81 | 94 | 94 |
| Not thrombosis | 85 | 56 | 52 | 52 |
| *After four days* | | | | |
| Thrombosis | | | | |
| All | 33 | 97 | 100 | 100 |
| Proximally | 22 | 95 | 100 | 100 |
| Distally | 11 | 100 | 100 | 100 |
| Not thrombosis | 64 | 39 | 36 | 34 |
| *After six days* | | | | |
| Thrombosis | | | | |
| All | 32 | 100 | 100 | 100 |
| Proximally | 22 | 100 | 100 | 100 |
| Distally | 10 | 100 | 100 | 100 |
| Not thrombosis | 63 | 38 | 37 | 35 |

Table IV *Number and percentage of correctly diagnosed legs/all legs in the modified FUT*

Results from CS I are included only if they were obtained before phlebography

| Findings at an independent review of phlebographies | CS I (*n*) | CS II (*n*) | CS I + II | |
|---|---|---|---|---|
| | | | *n* | % |
| *One hour after injection* | | | | |
| Thrombosis | | | | |
| All | 45/76 | 22/32 | 67/108 | 62 |
| Proximally | 35/40 | 16/22 | 51/62 | 82 |
| Distally | 10/36 | 6/10 | 16/46 | 35 |
| Not thrombosis | 64/82 | 41/63 | 105/145 | 72 |
| *After one day* | | | | |
| Thrombosis | | | | |
| All | 37/52 | – | 37/52 | 71 |
| Proximally | 21/21 | | 21/21 | 100 |
| Distally | 16/31 | | 16/31 | 52 |
| Not thrombosis | 46/63 | – | 46/63 | 73 |
| *After two days* | | | | |
| Thrombosis | | | | |
| All | 9/10 | 32/32 | 41/42 | 98 |
| Proximally | 4/4 | 22/22 | 26/26 | 100 |
| Distally | 5/6 | 10/10 | 15/16 | 94 |
| Not thrombosis | 18/22 | 26/63 | 44/85 | 52 |
| *After four days* | | | | |
| Thrombosis | | | | |
| All | 1/1 | 32/32 | 33/33 | 100 |
| Proximally | – | 22/22 | 22/22 | 100 |
| Distally | 1/1 | 10/10 | 11/11 | 100 |
| Not thrombosis | 0/1 | 23/63 | 23/64 | 36 |
| *After six days* | | | | |
| Thrombosis | | | | |
| All | – | 32/32 | 32/32 | 100 |
| Proximally | – | 22/22 | 22/22 | 100 |
| Distally | – | 10/10 | 10/10 | 100 |
| Not thrombosis | – | 23/63 | 23/63 | 37 |

Osm/kg) in 106 legs. Furthermore rephlebography was performed in 16 patients with normal veins at the first phlebography in whom strong signs of thrombosis developed after the examination with conventional contrast medium.

In CS I, phlebography was performed immediately after a pathological FUT. When FUT was normal, phlebography was performed as soon as possible (0–4 days after arrival). In CS II, phlebography was delayed 2 days (performed 2–4 days after arrival). In CS I, the routine report on phlebography was written by the general radiologist on duty, in CS II by one of three radiologists with a special interest in phlebography.

The results of plethysmography and FUT were given without knowledge of the results at phlebography. At the end of each series all phlebography films were carefully reviewed by one of us (U. A.), still unaware of all findings except phlebography. The results of these extra penetrating independent reviews were considered as the final diagnosis of thrombosis.

## RESULTS

The incidence of DVT was 50% in CS I and 34% in CS II (Table II). This difference ($p<0.02$) was mainly due to a decreased proportion of distal thrombosis in the second study.

*The modified FUT.* First, the diagnostic efficiency of three different three point criteria of thrombosis were compared (Table III). The results with the 5-4-4% criterion and the 4-4-4% criterion were identical during the first two days and very

Table V *Diagnostic efficiency at each measuring point in the modified ¹²⁵I fibrinogen technique evalu[ated] by phlebography*

| | Foot | Calf 1 | 2 | 3 | 4 | 5 | Knee | Thigh 1 | 2 | 3 | 4 | 5 | Mean v[alue] Calf | Th[igh] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sensitivity (%) | 71 | 76 | 80 | 97 | 93 | 93 | 95 | 86 | 95 | 94 | 93 | 83 | 88 | 90 |
| Specificity (%) | 83 | 68 | 68 | 59 | 62 | 62 | 65 | 76 | 76 | 78 | 79 | 79 | 64 | 78 |

similar later on The more rigorous one the 5 5 5% criterion detected thrombi more slowly than the other two criteria Specificity after 2 days was about the same for all three criteria In the sequel the FUT was regarded as positive when the 4-4-4% criterion or the 15% criterion were met

The ipsilateral criteria were used in 6 patients with symptoms in both legs Phlebography and FUT showed equal results in 3 of these patients (two were bilaterally normal and one had bilateral DVT) *In each of two other patients with bilateral* DVT at phlebography the ipsilateral criteria were correct in one leg In the sixth patient FUT was pathological up to the groin in both legs phlebo graphy could not be performed in the leg with most symptoms but showed DVT throughout the other leg Thus the ipsilateral criteria had correct results in 9 of 11 legs examined by phlebography

The sensitivity of FUT to DVT increased during the first two days (Table IV) Thus the test dis closed 62% of the thrombi one hour after ¹²⁵I fibrinogen injection 71% after one day and 98% after two days Thrombi with proximal extension (into or above popliteal vein) were detected early 82% were found after one hour and 100% after one day Distal thrombi (i e in foot and/or calf veins) were not revealed that early 35% were found after one hour 52% after one day 94% after two days a[nd] 100% after four days respectively

The sensitivity to extravascular fibrin (e g hematoma or inflammation) also seems to incre[ase] with time Thus the specificity of FUT to D[VT] decreased with time This decrease was alm[ost] completed after two days in CS II correctly ne[ga]tive results being found in 41 of 63 legs (65%) af[ter] one hour in 26 of 63 legs (41%) after two days [and] in 23 of 63 legs (37%) after four days The resu[lts] after six days were the same as after four days [In] CS I however specificity did not show any ch[ange/] decrease with time (calculations from Table IV g[ive] 78% after one hour 73% after one day and 8[?] after two days)

The extent of the thrombi was usually fairly w[ell] defined in the test after two days Thus in proxi[mal] thrombosis the top of the thrombus was on [an] average placed 3 2 cm lower than where it was se[en] at phlebography In distal thrombosis it was plac[ed] 5 3 cm higher than indicated by phlebograph[y] (Measurements performed after phlebography [in] CS I are not included in these calculations )

The diagnostic efficiency at each measuring po[int] after two days was also evaluated by phlebograp[hy]

Table VI *Number and percentage of correct plethysmographic diagnoses (all legs with original criteria in venous strain gauge plethysmography*

| Results at an independent review of phlebographies | Venous volume n | % | Venous emptying rate n | % |
|---|---|---|---|---|
| Thrombosis | | | | |
| All | 31/84 | 37 | 46/84 | 55 |
| Proximally | 23/42 | 55 | 35/42 | 83 |
| Distally | 8/42 | 19 | 11/42 | 26 |
| Not thrombosis | 74/86 | 86 | 78/86 | 91 |

Table VII *Number and percentage of corr[ect] plethysmographic diagnoses (all legs with alter[ed] criteria in venous strain gauge plethysmograp[hy] (decreased value in one or both of venous volum[e] and venous emptying rate)*

| Results at an independent review of phlebographies | Venous plethysmograph[y] n | % |
|---|---|---|
| Thrombosis | | |
| All | 53/84 | 63 |
| Proximally | 38/42 | 90 |
| Distally | 15/42 | 36 |
| Not thrombosis | 66/86 | 77 |

Table VIII *Reliability of the routine report on phlebography*

In CS I initial phlebographies and rephlebographies are included in a total of 196 examinations

| Results at an independent review of phlebographies | Correct findings in the routine report on phlebography | | | | | |
|---|---|---|---|---|---|---|
| | CS I | | CS II | | CS I + II | |
| | *n* | % | *n* | % | *n* | % |
| Thrombosis | | | | | | |
| All | 82/98 | 84 | 30/32 | 94 | 112/130 | 86 |
| Proximally | 37/37 | 100 | 22/22 | 100 | 59/59 | 100 |
| Distally | 45/61 | 74 | 8/10 | 80 | 53/71 | 75 |
| Not thrombosis | 91/98 | 93 | 61/63 | 97 | 152/161 | 94 |

(Table V) The sensitivity was almost equal on thigh and calf 90% and 88% on the average The specificity was higher on the thigh (78%) than on the calf (64%) The foot measurements showed the highest specificity (83%) but the lowest sensitivity (71%) of all points in the test

*Venous strain gauge plethysmography* The sensitivity to thrombosis using the original criteria (11) was 37% with venous volume and 55% with venous emptying rate (Table VI) The specificity was 86% and 91% respectively

With the altered criteria that were used in routine clinical work sensitivity was 63% to all thrombi or 90% to proximal and 36% to distal thrombi (Table VII) In patients with distal thrombi however also 36% of contralateral legs showed pathological results In patients with proximal thrombosis in the symptomatic leg 29% of the contralateral legs showed pathological results The specificity of altered criteria in legs without thrombosis was 77% (i e 23% pathological results) In the non symptomatic leg of these patients pathological results were found in 24%

*The routine report on phlebography* The report had correctly noted 86% of the thrombi that were found at the independent review of phlebography films (Table VIII) The sensitivity to proximal thrombi was 100% and to distal thrombi 75% (i e every fourth leg was missed in that group) The specificity was 94% The proportion of correct reports was significantly higher in CS II than in CS I ($p<0.05$)

## DISCUSSION

A diagnostic test should be evaluated on that particular group of patients for which it is intended (23) Accordingly the present study was performed in patients with suspected DVT Furthermore consecutive patients were studied since selection may bias evaluation In the present study 20% of the thrombi were located distally 24% extended proximally and 56% of the legs were normal Thus a broad spectrum of patients was studied Interpretation of test results and establishment of the final diagnosis were done independently The study therefore satisfies two important conditions proposed by Ransohoff and Feinstein (20) for the evaluation of diagnostic tests

In our experience more patients with a suspicion of DVT seek medical attendance during the winter than during other seasons Thus the higher incidence of DVT during the first study (Nov –April) than during the second (March–May) may reflect such a seasonal variation For unknown reasons it was mainly the incidence of distal thrombosis that differed in the two studies

*The modified FUT* Either high sensitivity or high specificity is required in a reliable screening test The present evaluation against phlebography showed a higher sensitivity to established DVT in the modified FUT (98%) than in most studies with conventional FUT Browse et al (5) found 71% Kakkar (12) 84% and Fridrich and Schmitt (8) 90% McIvor et al (14) report a sensitivity of 95% but that figure is probably due to their selection of patients with clinical evidence of DVT

In clinical routine early evaluation of patients with suspected DVT is necessary In conventional FUT early measurements have not been regarded useful Thus the first measurements have not been performed until 24 hours after giving the labelled fibrinogen except for Fridrich et al (9) who measured already after 6 hours Our measurements at

one hour after injection discovered 82% of proximal and 35% of distal thrombi The patients with proximal thrombi not detected at the one hour examination usually had extensive edema causing attenuation of the $^{125}$I activity If no extensive edema exists normal FUT results at one hour exclude proximal thrombosis for practical purposes

During the first two days fibrinogen continued to accumulate not only intravascularly (in thrombosis) but also extravascularly (in hematoma or inflammation) *In other words the specificity in patients* without DVT decreased with time This was not apparent in CS I where phlebography was performed immediately after a pathological FUT and subsequent measurements had to be excluded due to thrombotic complications caused by the contrast medium (Table IV) Conditions with hematoma or inflammation were often found to simulate thrombosis This explains the low specificity of FUT in suspected DVT in the present as well as in most previous studies where values of 85% (5) 54% (12) and 45% (14) have been found However Fridrich and Schmitt (8) found a high specificity (91%) but they selected patients without wounds and hematoma

The extent of the thrombi was fairly well defined with the modified FUT on the thigh as well as on the calf Furthermore the diagnostic efficiency was about the same on thigh points as on calf points This finding contradicts the view held by previous authors that thrombi at proximal thigh are difficult to find with FUT (4 7 14) Fridrich et al (9) studied the sensitivity at each measuring point and found 30–35% *sensitivity on proximal thigh* 34–36% on distal thigh 33% at popliteal vein and 52–59% on calf The higher sensitivity in the present study is probably due to the modified technique e g special precautions to empty pooled venous blood high count rate at each measuring point dorsal measurements in the knee region and finally foot measurements

The concept of a 3 point criterion (5) seems to be useful in the early diagnosis of DVT In the present study however the 4-4-4% criterion and the 5-4-4% criterion detected DVT more rapidly than the 5 5 5% criterion During the first two days the results with the 5-4-4% and the 4-4-4% criteria were identical and for simplicity the latter criterion was *preferred*

The practical conclusion from Table III is that with any one of the two latter criteria measurements during two days are sufficient to exclude DVT

Browse et al (5) found that the accuracy of the test was unaffected by the administration of heparin Their view was not contradicted in the present series but only few patients received heparin before injection of $^{125}$I fibrinogen (anticoagulants were usually given after the first pathological FUT i e on the first day in most cases with DVT)

*Venous plethysmography* showed high sensitivity *and specificity in a study of non consecutive pa*tients (11) In the present study of consecutive patients however the method was found to lack both sensitivity and specificity even with altered criteria The low sensitivity may be due to various reasons As a rule the method cannot detect foot or calf thrombi Furthermore even proximal thrombosis may not be detected as in partial occlusion (e g with floating thrombi) and in patients with double veins or varicose veins Varicosities usually give high plethysmographic values that decrease to normal if DVT occurs The lack of specificity may also be due to venous stasis as in postthrombotic limbs in congestive heart failure and in external compression upon the veins

The results obtained by Hallbook and Gothlin (11) and in the present study are quite different as regards the diagnostic efficiency of plethysmography despite close similarity in technique and criteria In addition to what was mentioned above the difference may be explained by the varying types of patients included Thus Hallbook and Gothlin found distal thrombosis in only 5 (out of 36 patients whereas in the present study 52 (out of 117) patients had distal thrombosis It was found in the present study that plethysmography is not reliable as a screening test for distal thrombi Nevertheless the method might still be useful for studies of venous function e g to evaluate the response to treatment of proximal thrombosis

*The routine report on phlebography* Phlebography films are often difficult to interpret for physicians without special experience in diagnostic radiology Superficial and deep veins often intermingle on the same film especially in the calf region Also well trained general radiologists were found to have difficulties in this region Thus the initial routine report missed every fourth patient *with distal thrombi detected at the later independ*ent review When CS I and CS II were considered separately however the best results were found

ith CS II reports that were written by one of three radiologists with special interest in angiology. The modified FUT has some advantages: a normal result after two days excluded DVT significantly more reliably than the routine report on phlebography ($p<0.05$).

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## REFERENCES


1. Albrechtsson U & Olsson C-G. Thrombotic side-effects of lower limb phlebography. Lancet 1 723 1976
2. — Thrombosis following phlebography with ionic and non-ionic contrast media. Acta Radiol 20 46 1979
3. — Thrombosis after phlebography. A comparison of two contrast media. Card Vasc Radiol 2 9 1979
4. Browse N L. The 125I fibrinogen uptake test. Arch Surg 104 160 1972
5. Browse N L, Clapham W F, Croft D N, Jones D J, Lea Thomas M & Olwen Williams J. Diagnosis of established deep vein thrombosis with the 125I fibrinogen uptake test. Br Med J 4 325 1971
6. Darte L, Olsson C-G & Persson R B R. Rapid detection of deep vein thrombosis with 99Tcm plasmin using hand detector or scintillation camera. Proc XIV Int Ann Meeting Soc Nucl Med Berlin 1976. In: Nuklearmedizin I 524. Medio-Informationsdienst Berlin 1978
7. Flanc C, Kakkar V V & Clark M B. The detection of venous thrombosis of the legs using 125I labelled fibrinogen. Br J Surg 55 742 1968
8. Fridrich R & Schmitt H E. Zur Diagnose von Venenthrombosen mit nuklearmedizinischen Verfahren. Med Welt (N F) 26 1960 1975
9. Fridrich R, Schmitt H E, Madar G & Widmer L K. Radiofibrinogen-Test bei etablierter Venenthrombose. Vasa 3 446 1974
10. Haeger K. Den kliniska trombosdiagnosens (o)tillförlitlighet. Läkartidningen 62 1067 1965
11. Hallbook T & Gothlin J. Strain gauge plethysmography and phlebography in diagnosis of deep venous thrombosis. Acta Chir Scand 137 37 1971
12. Kakkar V V. The diagnosis of deep vein thrombosis using the 125I fibrinogen test. Arch Surg 104 152 1972
13. Lundh B. Personal communication
14. McIvor J, Anderson D R & Dovey P. Comparison of 125I labelled fibrinogen uptake and venography in the detection of recent deep-vein thrombosis in the legs. Br J Radiol 48 1013 1975
15. Negus D, Pinto D J, Le Quesne L P, Brown N & Chapman M. 125I labelled fibrinogen in the diagnosis of deep-vein thrombosis and its correlation with phlebography. Br J Surg 55 835 1968
16. Nicholaides A N, Kakkar V V, Field E S & Renney J T G. The origin of deep vein thrombosis: a venographic study. Br J Radiol 44 653 1971
17. Olsson C-G. A modified 125I fibrinogen technique for thrombus detection in the whole leg. Scand J Clin Lab Invest 39 677 1979
18. Olsson C-G, Albrechtsson U, Darte L & Persson R B R. 99Tcm plasmin for rapid detection of deep vein thrombosis. J Nucl Med. To be published
19. Rabinov K & Paulin S. Roentgen diagnosis of venous thrombosis in the leg. Arch Surg 104 134 1972
20. Ransohoff D F & Feinstein A R. Problems of spectrum and bias in evaluating the efficacy of diagnostic tests. N Engl J Med 299 926 1978
21. Westling H. Personal communication
22. Williams W J. Venography (editorial). Circulation 47 220 1973
23. Wulff H R. Diseases—the vehicles of clinical experience. In: Rational diagnosis and treatment pp 73–75. Blackwell Scientific Publications, Oxford 1976

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# Methenamine-Hippurate and Bacteriuria in the Geriatric Patient with a Catheter

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ABSTRACT Oral treatment with methenamine-hippurate (MH) in patients with an indwelling catheter has been found to reduce the need of frequent catheter exchange and the number of symptomatic infections Bacteriuria however, persists during MH treatment. The hypothesis that the therapeutic effect is due to a reduction in the number of bacteria or a change in the pattern of strains was tested in a cross-over study (2×6 weeks) MH treatment 1 g twice daily, was compared to control periods in 52 patients. The majority of quantitative and qualitative bacterial cultures at 2 week intervals yielded 2–4 strains. Of the bacterial isolates, 50% were found on 4–6 occasions out of 6 possible MH treatment had no significant influence on the pattern of various strains. A 30% decrease in the mean total bacterial count during MH treatment did not reach statistical significance ($p=0.07$) It is suggested that prevention of catheter complications during MH treatment may be due to a physiochemical action on salt formation rather than a direct antibacterial effect

*Key words* methenamine hippurate bacteriuria indwelling catheter

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The use of an indwelling catheter in the care of chronically ill patients causes persistent bacteriuria. Catheter treatment is frequently associated with problems such as incrustation and obstruction of the catheter leakage of urine formation of bladder stones foul smell in the ward and episodes of symptomatic urinary tract infection Antimicrobial drugs should not be used in the treatment of bacteriuria per se as the bacteria are likely to remain or be replaced by resistant strains Despite the disadvantages indwelling catheters have to be used in a large number of patients Various means to reduce the burden of complications therefore deserve investigation

Oral administration of methenamine hippurate (MH) has been used with varying success in the treatment of symptomatic or asymptomatic urinary tract infections and is considered suitable as prophylaxis (2 3 7 9 11 15 17) MH administration will not encourage resistant bacterial strains and few side-effects have been reported In patients with an indwelling catheter bacteriuria will persist during MH treatment (7 14) However the need of catheter exchanges diminished significantly and the incidence of symptomatic urinary tract infections decreased (14) This might have been due to a reduction of the urinary bacterial count though such an effect could not be demonstrated by a semi-quantitative dip slide technique

The aim of this study was to investigate further the effects of MH treatment on the number of bacteria and the pattern of bacterial strains in the urine of patients with an indwelling catheter

## PATIENTS AND METHODS

Fifty-two patients were selected from seven wards in a hospital for chronically ill patients Their mean age was 83 years and 85% were females All had indwelling catheters (Foley no 14–18) which were routinely exchanged every four weeks Patients with rapidly deteriorating illness or with S-creatinine values of ≥140 μmol/l were excluded None had received antibiotic treatment during the two weeks preceding the study

### *Design of the study*

Group A consisted of 19 patients already on MH treatment 1 g twice daily They remained on this treatment for 6 weeks and were then observed for 6 weeks without treatment Group B consisting of 33 patients was not treated with MH during the initial 6 weeks but with 1 g MH twice daily for the following 6 weeks. Complications

*Abbreviations* MH = methenamine-hippurate MH period = methenamine-hippurate period C period = control period CLED – cystine-lactose-electrolyte-deficient

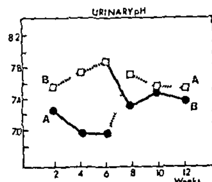



*Fig 1* Mean urinary pH at each sampling during MH periods (●—●) and C periods (□ □) in groups A and B

in conjunction with the catheters and exchanges were recorded during the 12 weeks of study Irrespective of MH treatment antibiotics were prescribed when indicated Urine samples for bacterial culture counting and classification and for pH determination were collected at 2 week intervals i e on three sampling occasions during treatment (MH period) and three during the control period (C period)

*Sampling*

Sampling took place before breakfast after the catheters had been plugged for 3 hours The first portion of urine was discarded and the second was collected in a sterile tube The samples were immediately cooled and kept at +4 C until cultured (within 5 hours)

*Urinary pH* was determined immediately after sampling using indicator strips (Lyphan accuracy ± 0 2 pH units)

*Bacteriological techniques*

The urine was diluted 1/100 and 1/1 000 in sterile 0 9% saline Culture media used were cystine lactose electrolyte-deficient (CLED) agar (Oxoid England) and sodium azide agar Cultures were performed in duplicate on each medium from undiluted and diluted urine (12 plates for each sample) using a 0 002 ml calibrated loop (8) All plates were incubated at 37°C Gram negative bacteria were counted on CLED agar after 24 hours and gram positive bacteria on sodium azide agar after 48 hours The bacterial concentrations were estimated from plates with 20–200 colonies Occasionally it was necessary to depart from these limits Only cultures yielding ≥10000 bacteria/ml were included in the study and submitted f identification Beta streptococci were classified by t co-agglutination technique (5) other species by routi methods The laboratory was not informed whether t patients were on MH treatment or not

Table I *Patients treated with antibiotics during MH and C periods*

| | Urinary tract infection | | Other infections | |
|---|---|---|---|---|
| | MH period | C period | MH period | C period |
| Group A ($n=19$) | 0 | 2 | 2 | 0 |
| Group B ($n=33$) | 0 | 3 | 3 | 3 |
| Total | 0 | 5 | 5 | 3 |

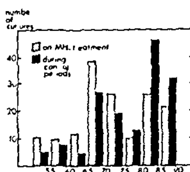



*Fig 2* Number of specimens with different urinary p (Urine was incubated for 3 hours in the bladder )

## RESULTS

*Need of antibiotic treatment* (Table I)

Symptomatic urinary tract infections required treatment during MH periods whereas 2 patients group A and 3 in group B received such treatme during the C periods For other reasons (glute perineal and periurethral abscesses erysipelas a respiratory tract infections) 2 patients in group and 3 in group B were treated with antibiotics du ing the MH periods and 3 in group B during the period

*Need of exchange of the indwelling catheter*

The frequency of changing the indwelling cathet due to incrustation is shown in Table II Patien who had been treated with antibiotics or bladd lavage and those who had repeatedly withdraw their catheters were excluded This resulted in reduction of about one third of the patients in bo groups The rate of catheter exchange was 0 5 week during C periods and 0 40/week during M periods This difference was not statistically si

Table II *Number of patients with catheter exchange due to incrustation*

| | MH period | C period |
|---|---|---|
| Group A ($n=13$) | 40 | 59 |
| Group B ($n=22$) | 44 | 61 |
| Total | 84 | 120 |

Table III *Number of patients with various bacteria in single or repeated urine culture*
6 cultures in each of 52 patients

| | Number of times cultured from the same subject | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | All 6 times |
| E coli | 7 | 5 | 3 | 5 | 6 | 11 | 15 |
| Proteus | 10 | 5 | 5 | 5 | 6 | 11 | 10 |
| *Enterococci* | 7 | 7 | 3 | 8 | 12 | 7 | 8 |
| Providencia | 24 | 5 | 7 | 2 | 7 | 3 | 4 |
| *β* Streptococci group B | 32 | 7 | 3 | 3 | 4 | 3 | 0 |
| Pseudomonas | 40 | 1 | 1 | 6 | 2 | 1 | 1 |
| Klebsiella | 40 | 4 | 3 | 0 | 1 | 0 | 4 |
| α Streptococci | 38 | 8 | 2 | 3 | 0 | 1 | 0 |
| Staphylococcus aureus | 43 | 4 | 2 | 1 | 1 | 1 | 0 |
| Staphylococcus epidermidis | 43 | 9 | 0 | 0 | 0 | 0 | 0 |
| Edwardsiella | 50 | 2 | 0 | 0 | 0 | 0 | 0 |
| Enterobacterium hafnia | 51 | 1 | 0 | 0 | 0 | 0 | 0 |
| | | 58 | 29 | 33 | 39 | 38 | 42 |

nificant as 5 of the patients contributed more than 50% of the total difference Table II underestimates however the effect of MH treatment because of the above mentioned exclusions

*Urinary pH*

The mean pH for the six sampling occasions calculated for groups A and B separately is shown in Fig 1 Treatment with MH was associated with a small decrease in mean pH from 7 7 to 7 4 Group B (not treated with MH previously) had a slightly higher pH than group A As shown in Fig 2 most urine samples had a pH around 7 or higher and a pH below 6 5 was rarely recorded

*Bacterial culture*

Fig 3 shows that urine cultures usually yielded 2–4 strains of bacteria Table III shows that 50% of the bacterial isolates were reproduced 4–6 times and that E coli Proteus Enterococci and Providencia tended to be common and persistent

Table IV shows how often various bacteria were found in the cultures and the fraction of patients who had harboured them at least once The table also displays how often during MH and C periods various bacteria had been the dominant microorganism in a culture specimen and the dominant organism during antibiotic treatment Negative findings for bacterial growth (1 6% of all cultures) were obtained only during antibiotic treatment No significant differences in the occurrence of various bacteria were found between the MH and C periods Further the pattern of cultured strains was the same in patients in group A as in group B

A quantitative evaluation of bacteriuria was performed on the 14 patients in group A and 24 in group

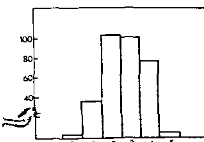


*Fig 3* Number of occasions on which a culture contained 0–5 different bacterial strains

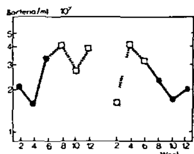


*Fig 4* Mean number of total bacteria/ml urine at each sampling during MH periods (●—●) and C periods (□ □) Group A to the left group B to the right S E M is indicated by shadowed areas

Table IV *Various bacteria in 312 urine cultures (156 during MH therapy) from 52 patients*

| | Per cent of all 312 cultures | Per cent of 52 patients | Dominating organism: Per cent of 156 cultures during MH period | Dominating organism: Per cent of 156 cultures during C period | Per cent of 20 cultures during antibiotic therapy |
|---|---|---|---|---|---|
| E coli | 62 6 | 86 4 | 40 4 | 33 4 | 10 |
| Proteus | 54 2 | 80 6 | 25 6 | 31 5 | 35 |
| Enterococci | 53 9 | 86 4 | 6 4 | 8 3 | 5 |
| *Providencia* | *29 5* | 53 8 | *14 7* | *18 0* | *0* |
| *β Streptococci* group B | 17 0 | 38 4 | 1 9 | 0 6 | 0 |
| Pseudomonas | 12 8 | 23 0 | 4 5 | 1 3 | 10 |
| Klebsiella | 12 2 | 23 0 | 0 6 | 2 6 | 0 |
| *α Streptococci* | 8 3 | 26 9 | 1 9 | 0 6 | 5 |
| Staphylococcus aureus | 6 4 | 17 3 | 1 9 | 1 0 | 0 |
| Staphylococcus epidermidis | 2 9 | 17 3 | 0 0 | 0 6 | 5 |
| No bacterial growth | 1 6 | 9 6 | 0 6 | 1 9 | 30 |
| *Edwardsiella* | *0 6* | 3 8 | 0 0 | *0 6* | *0* |
| Enterobacterium hafnia | 0 3 | 1 9 | 0 6 | 0 0 | 0 |

B who had not received antibiotic treatment during the study As expected the range (±2 S D ) for total bacterial counts was wide 9×10 –6×10 bacteria/ml Log scale mean values on each of the six sampling occasions were calculated for groups A and B (Fig 4) The mean total bacterial count was 2×10 /ml during MH periods and $3\times10^7$/ml during C periods The difference between the total bacterial counts (mean of three values) during C periods and MH periods was calculated for each patient The mean value (± S D ) for the differences in all these paired observations expressed as log $10^6$ bacteria/ml was 0 15±0 47 ($p$=0 07)

## DISCUSSION

Treatment with MH has been reported to have beneficial clinical effects in patients with an indwelling catheter The frequency of changing catheters on demand was significantly reduced in a cross over study (2×4 months) of 22 patients receiving 1 g MH twice daily (14) In an independent double blind study of 36 patients receiving 1 mg MH three times daily Norberg et al (12) reported a significant increase in the mean catheter life time from 8 to 14 days In the present study incrustation of catheters also occurred less frequently during MH periods

A reduced incidence of catheter obstruction is in itself likely to be associated with fewer episodes of symptomatic urinary tract infections The frequency of clinical infections was actually reported to be reduced in a previous study (14) 4 episodes in the MH group and 16 during a comparable control period The 5 cases of infection observed in this study all occurred during control periods Catheter problems were more common in group A which to some extent was a preselected group of patients with a previous history of urinary tract symptoms When the patients in group A were switched over to control periods the need of repeated bladder instillations and lavage in one patient urinary gravel problems in 2 and symptomatic urinary tract infection in 2 patients developed within 10 days

The common opinion is that oral urinary tract antiseptics may decrease the number of viable bacteria in the urine (16) MH is believed to act through the acidifying and bacteriostatic effects of hippuric acid in combination with the antibacterial methenamine which is hydrolysed to formaldehyde in an acid environment Despite clinical effects of MH treatment all our 52 patients had persistent bacteriuria In 38 patients evaluated quantitatively the mean bacterial counts decrease by 30% or 10 bacteria/ml This decrease did not reach statistical significance ($p$=0 07) Thus previous difficulties in demonstrating significant effects of oral urinary antiseptics on mean bacterial counts in catheter patients are confirmed (18) Although urinary pH tended to be lower during MH treatment it was almost always higher than 6 5 The alkaline pH may be due to the incubation of urine with urea splitting bacteria in the bladder prior to sampling The urine originally formed in the kidneys is bound to have

been more acid possibly facilitating the antibacterial effects of MH in the upper urinary tract

The pattern of bacterial strains in the urine was similar during MH and C periods The frequency and distribution of various bacteria were similar to the findings of other studies (1 4) although Providencia seemed to be a locally important organism (10 19) Whereas the variability of the bacterial findings in geriatric patients has been stressed by others (1) we found that most patients had a rather constant pattern of bacterial strains over the three months of study

There seems to be reasonably good evidence that treatment with MH can reduce some of the complications of catheter treatment such as incrustation and obstruction MH may due to an antibacterial effect prevent spread of infection to the upper urinary tract However it can be concluded that the main action of MH in catheter patients is neither antibacterial nor acidifying The clinical effect may instead be due to physiochemical alterations in the urine which inhibit the precipitation of salts The processes of salt formation seem to deserve further investigation For example it has been reported that hippuric acid for each pH level can increase the solubility of both inorganic and organic components of urine (6)

## REFERENCES


1 Alling B Brandberg Å Seeberg S & Svanborg A Aerobic and anaerobic microbial flora in the urinary tract of geriatric patients during long term care J Infect Dis 127 34 1973

2 Almgård L E Ericsson H & von Garrelts B Infection prophylaxis of urine in connection with instrumental investigation Lakartidningen 69 6146 1972

3 Andelman M B Control of bacteriuria in geriatric populations Ill Med J 133 273 1968

4 Brumfitt W & Reeves D S Recent developments in the treatment of urinary tract infection J Infect Dis 120 61 1969

5 Christensen P Kahlmeter G Jonsson S & Kronvall G New method for the serological grouping of streptococci with specific antibodies adsorbed to protein A containing staphylococci Infect Immun 7 881 1973

6 Elliot J S & Ausebio E Calcium oxalate solubility the effect of inorganic salts urea creatinine and organic acids Invest Urol 3 72 1965

7 Gerstein A R Okun R Gonick H C Wilner H I Kleeman C R & Maxwell H M The prolonged use of methenamine hippurate in the treatment of chronic urinary tract infection J Urol 100 767 1968

8 Hoeprich P D Culture of the urine J Lab Clin Med 56 899 1969

9 Kasanen A Kaarsalo E Hiltunen R & Soini V Comparison of longterm low-dosage nitrofurantoin methenamine hippurate trimethoprim and trimethoprim sulphamethoxazole on the control of recurrent urinary tract infection Ann Clin Res 6 285 1974

10 Klastersky J Bogaerts A M Noterman J van Laer E Deneau D & Mouawad E Infections caused by Providence bacilli Scand J Infect Dis 6 153 1974

11 Nilsson S Long term treatment with methenamine hippurate in recurrent urinary tract infection Acta Med Scand 198 81 1975

12 Norberg A Gippert A Norberg B & Parkhede U Profylaktisk behandling med methenaminhippurat av urinvagsinfektion hos kateterbarare En randomiserad dubbelblind studie Acta Soc Med Suec 87 617 1978

13 Norberg B Norberg A Parkhede U & Gippert H The effect of short term high-dose treatment with methenamine hippurate of urine infection in geriatric patients with indwelling catheters IV Clinical evaluation Eur J Clin Pharmacol In press 1979

14 Norrman K & Wibell L Treatment of methenamine hippurate in the patient with a catheter J Intern Med Res 4 115 1976

15 Petersen S Long term prophylaxis with methenamine hippurate in girls with recurrent urinary tract infections Acta Paediatr Scand 67 597 1978

16 Schaberg D R Weinstein R A & Stamm W E Epidemics of nosocomial urinary tract infection caused by multiple resistant gram negative bacilli Epidemiology and control J Infect Dis 133 363 1976

17 Seneca H Zinsser H H & Peer P Chemotherapy of chronic urinary tract infections with methenamine hippurate J Urol 97 1094 1967

18 Vainrub B & Musher D M Lack of effect of methenamine in suppression of or prophylaxis against chronic urinary infection Antimicrob Agents Chemother 12 625 1977

19 Whitley G R Penner J L Stewart I O Stokan P C & Hinton N A Nosocomial urinary tract infections caused by two O serotypes of Providencia stuartii in one hospital J Clin Microbiol 6 551 1977

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# Metabolic Control of Diabetes Mellitus during Routine Management at an Out-Patient Department

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ABSTRACT The current study comprised 376 living and 140 deceased diabetic patients A majority of the patients had received oral hypoglycemic agents (tablets) or insulin, while a modified diet alone had been prescribed for a minority The mean age of the tablet treated patients (67 and 73 years for those now alive and deceased, respectively) was in general 6–10 years higher than that of insulin treated patients The mean morning blood glucose level ranged between 8.3 and 10 5 mmol/l A considerable number of the tablet treated patients were overweight and hypertriglyceridemic About one third of the patients in both groups were considered to be in a fairly good state of metabolic control arbitrarily defined as a urinary glucose excretion of less than 110 mmol/24 h, a relative body weight of less than 115% and a serum triglyceride level below 2 0 mmol/l Cardiovascular disease was the cause of death in 60–70% of the patients Patients with clinical evidence of atherosclerosis did not differ from age matched pair mates without signs of macroangiopathy with regard to hyperglycemia and hyperglucosuria However, the prevalences of hypertriglyceridemia hypertension and overweight tended to be higher in the former It is concluded that factors of importance for development of atherosclerosis in a non diabetic population should be considered in the treatment of diabetes mellitus

*Key words* diabetes mellitus glucose overweight serum triglycerides macroangiopathy microangiopathy causes of death

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Diabetes mellitus (DM) is known to be associated with premature development of macro- and micro angiopathy It has been suggested (4) but not yet generally agreed (21) that proper control of the blood glucose level will postpone the development of vascular complications Evidence supporting this concept in DM of juvenile type was reported recently by Deckert et al (6) However the criteria of effective control were very strict and only a small proportion of the patients fulfilled them The possibility of influencing cardiovascular disease in DM of the adult onset type by reducing blood glucose has been questioned Thus a long term prospective study revealed only minor differences in the incidences of fatal and non fatal events (myocardial infarction cerebrovascular disease) among placebo- and insulin treated patients in spite of marked differences in blood glucose levels (12) These findings focus interest on current knowledge about the mechanisms responsible for the development of various forms of angiopathy As the Framingham study clearly showed the atherosclerotic lesion is of multifactorial origin the most potent risk factors (besides age) being hypertension hypercholesterolemia and smoking followed by glucose intolerance and overweight (10) A further risk factor identified in other studies is hypertriglyceridemia (5 16) As the risk factors found in a general population are valid also for diabetic patients (11) it appears that metabolic control in DM should have a broader definition than just blood glucose reduction

The current investigation was undertaken to evaluate how routine DM care functions in a modern out patient department with regard to the various risk factors mentioned above Judging from the prevalences of hyperglycemia hyperlipoproteinemia and overweight only a small proportion of the patients were in a fairly good state of metabolic control As a conceivable consequence the occurrence of cardiovascular disease turned out to be above normal

*Abbreviations* DM diabetes mellitus RBW = relative body weight IHD ischemic heart disease group D = diet treated patients group T = tablet treated patients group I = insulin treated patients

Table I [illegible] (L) [illegible] deceased (D) [illegible]

| | | Group D | Group T | Group I |
|---|---|---|---|---|
| N | L | ?1 (11 ♀) | 15? (97 ♀) | 03 (1?3 ♀) |
| | D | 10 (6 ♀) | 63 (33 ♀) | 67 (30 ♀) |
| Age | | | | |
| At death | L | 57 3 3 | 67 0 9 | 57 1 ? |
| | D | 67+3 5 | 73+0 9 | 67 1 3 |
| At onset of DM | L | 55+3 4 | 59 0 9 | 4?+1 4 |
| | D | 61+4 0 | 63+1 3 | 5?+1 7 |
| RBW (%) | L | 1??+5 1 | 117+1 6 | 108+1 6 |
| | D | 1?5+9 6 | 11?+? 8 | 113±? 5 |
| Blood glucose (mmol/l) | L | 8 3+0 4 | 10 4+0 3 | 10 5+0 3 |
| | D | 7 7 0 8 | 10 3+1 0 | 10 1+0 5 |
| Serum triglycerides (mmol/l) | L | ? ? 0 4 | ? 5+0 ? | 1 9+0 1 |
| | D | | ? 3 0 ? | ? ?+0 4* |
| Serum cholesterol (mmol/l) | L | 5 9+0 3 | 6 3+0 1 | 6 0 0 1 |
| | D | 7 1+0 7 | 6 ?+0 4 | 6 6+0 4 |

Data a[illegible]lable for 0 pat[illegible]ents * Data ava[illegible]lable for 3? pa[illegible]ents

## SUBJECTS AND METHODS

*DM care at St Erik's Hospital* St Erik's Hospital is a community hospital in Stockholm responsible for the hospitalized care of about 1 0000 citizens A considerable proportion of patients with DM are treated at the Diabetic Out Patient Department Milder cases are generally referred to district physicians Information about DM self care complications dietary and pharmacologic treatment is given by the physician both at the Out Patient Department and at the Day Care Unit A diet cian working exclusively with diabetic patients is mainly responsible for the instruction in diet and nutrition From her the patients receive individual information on several occasions No formal educational program and only a few audiovisual illustrations are used The patients are supplied with some literature and written prescriptions about the recommended dietary regimen

As a general policy the patients are prescribed a diet that for those who are overweight is designed for weight reduction Otherwise the diet is similar to the standard hospital diabetic diet in which 45% of the energy is supplied as carbohydrate 35% as fat and 0% as protein Insulin is given without delay to insulin dependent patients Antihyperglycemic oral drugs are generally prescribed to patients who display inadequate control of glucose homeostasis after 1 ? months of treatment with diet alone Sulfonylurea is the primary drug When required this therapy is supplemented with metformin

### Patients

All patients visiting the Diabetic Out Patient Department during Jan April 1977 participated in the study 21 of them were treated with diet alone (group D) 15? with diet and oral hypoglycemic tablets group T) and 03 with diet and insulin (group I) The mean age of group T (67 years) was about 10 years higher than that of the other groups (Table I) Altogether 43 patients (all in group I) were considered to have DM of juvenile type (onset before [illegible] years of age)

Another series was made up of deceased patients comprising all ex[illegible] visitors who have died since the start of the Diabetic Out Patient Department in 197? Of these 10 had been treated with diet alone 63 with tablets and 67 with insulin At the time of death they were on the average 6– years older than the living patients (Table I)

### Protocol

Data on histories as well as physical and laboratory findings were obtained from the records and autopsy protocols (available for 95% of the deceased patients) and organized for computer analysis The definitions used were as follows *Relative body weight* (RBW) calculated according to Lindberg et al (13) *Hypertension* scored positive when antihypertensive drugs were prescribed by the patient's physician *Retinopathy* routine ophthalmoscopy by an ophthalmologist no grading of severity *Nephropathy* persistent proteinuria exceeding trace amounts and/or serum creatinine above 1 0 µmol/l *Neuropathy* absence of patellar and/or Achilles reflexes and/or loss of peripheral sense of vibration *Thyroid disease* non toxic goitre hyper or hypothyroidism thyroiditis *Angina pectoris* retrosternal pain with or without radiation to the left shoulder and arm aggravated by exercise and/or emotion relief after sublingual nitroglycerin and/or a few minutes rest *Myocardial infarction* hospital diagnosis based on clinical picture changes in serum enzymes (CPK ASAT and ALAT) and ECG In a few patients with no history of myocardial infarction the diagnosis w[illegible]

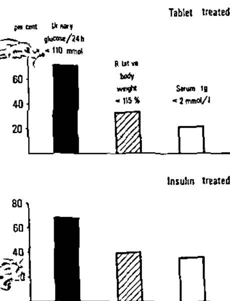



*Fig 1* Percentage distribution of patients in a state of metabolic control ■ = Urinary glucose <110 mmol/24 h ▨ = urinary glucose <110 mmol/24 h and RBW <115% □ = urinary glucose <110 mmol/24 h RBW <115% and serum triglyceride levels <2 0 mmol/l

based on ECG changes indicating earlier myocardial infarction *Cerebrovascular lesion* hospital diagnosis based on history and neurological findings *Intermittent claudication* pain or stiffness in the calf on walking relief after a few minutes rest

Blood samples for chemical analyses were drawn in the morning Most chemical determinations of blood were performed with a 20 channel autochemist (AGA Sweden) Blood and urinary glucose was analyzed with a glucose dehydrogenase method (Gluc DH Merck Darmstadt F R Germany)

*Statistical analysis*

Data are presented as mean ± S E M The significance of differences was evaluated by the χ test with Yates s correction and Student s paired and unpaired *t* tests (22)

## RESULTS

*Living patients*

*Glucose in blood and urine* Blood glucose averaged 8 3 10 4 and 10 5 mmol/l in groups D T and I respectively The urine contained no detectable amounts of glucose in 67% of group D 36% of group T and 22% of group I patients A urinary glucose excretion exceeding 110 mmoles was recorded for 5% (group D) 26% (group T) and 37% (group I) of the patients

Table II *Percentage prevalence of vascular disease and other complications in living (L) and deceased (D) patients*

| | | Group | | |
|---|---|---|---|---|
| | | D | T | I |
| Intermittent claudication | L | | 11 | 5 |
| | D | | 5 | 15 |
| Leg ulcer | L | | 3 | 5 |
| | D | | 10 | 21 |
| Angina pectoris | L | 14 | 22 | 8 |
| | D | | 21 | 18 |
| Myocardial infarction | L | | 13 | 6 |
| | D | 30 | 56 | 45 |
| Cerebrovascular disease | L | | 6 | 6 |
| | D | 40 | 27 | 19 |
| Hypertension | L | 43 | 36 | 17 |
| | D | 70 | 44 | 28 |
| Neuropathy | L | 24 | 38 | 40 |
| | D | 30 | 40 | 37 |
| Retinopathy | L | | 13 | 31 |
| | D | 10 | 33 | 65 |
| Nephropathy | L | 5 | 18 | 17 |
| | D | 40 | 25 | 37 |
| Thyroid disease | L | 14 | 11 | 13 |
| | D | | 10 | 6 |

*Body weight* The mean RBW was 122% (range 84–169) in group D 117% (range 76–185) in group T and 108% (range 72–182) in group I It exceeded 115% in 67 53 and 32% of the patients in groups D T and I respectively

*Serum lipids* The mean cholesterol level was within the normal range in all groups whereas the average triglyceride level was above the upper normal limit (2 0 mmol/l) in groups D and T (Table I)

*Metabolic control* A fairly good state of metabolic control was defined arbitrarily as urinary glucose excretion of less than 110 mmol/24 h RBW of less than 115% and serum triglyceride level below 2 0 mmol/l The results obtained by separating the patients into subgroups with regard to these variables indicated that most patients were defective in some way The largest subgroup of group T (26%) was characterized by overweight hypertriglyceridemia and urinary glucose excretion of less than 110 mmol/day Only 22% of the patients fulfilled all criteria for adequate metabolic control The patients in group I were less overweight Altogether

Table III *Characteristics of patients with macroangiopathy (MA) compared with age matched (am) and not age matched subjects without evidence of MA*

| | Group T | | | Group I | | |
|---|---|---|---|---|---|---|
| | | No MA | | | No MA | |
| | MA | *am* | *not am* | MA | *am* | *not am* |
| N | 52 (29 ♀) | 52 (36 ♀) | 48 (30 ♀) | 34 (23 ♀) | 34 (22 ♀) | 135 (79 ♀) |
| Age (y) | 70.3±1.2 | 70.3±1.3 | 60.2±1.5 | 67.9±1.6 | 67.7±1.5 | 51.2±1.6 |
| Duration of DM (y) | 8.6±0.8 | 8.8±0.7 | 7.7±1.0 | 15.5±1.6 | 15.4±1.9 | 14.1±0.8 |
| Blood glucose (mmol/l) | 10.0±0.4 | 10.4±0.4 | 11.0±0.5 | – | – | – |
| Urinary glucose < 110 mmol/ 24 h (% of cases) | 75 | 71 | 77 | 76 | 74 | 59 |
| RBW (%) | 118+2 | 114±2 | 118±3 | 119±5 | 111±3 | 105±2[d,f] |
| Serum triglyceride (mmol/l) | 3.0±0.3 | 2.1±0.2 | 2.3±0.2 [f] | 2.6±0.3 | 1.8±0.2 | 1.7±0.1 [f] |
| Serum cholesterol (mmol/l) | 6.4±0.2 | 6.4±0.2 | 6.4±0.2 | 6.3±0.2 | 5.8±0.2 | 5.8±0.2 |
| Hypertension (% of cases) | 44 | 40 | 19[a,g] | 35 | 15 | 13 [g] |
| *Fairly good control* (% of cases) | 21 | 27 | 21 | 17 | 44 [g] | 40[a,g] |

Comparisons with patients with MA [a] $p<0.05$ [b] $p<0.01$ [c] $p<0.005$ [d] $p<0.001$
Statistical tests [e] paired *t* test [f] unpaired *t* test [g] $\chi^2$ test with Yates's correction

34% fulfilled the criteria for being in fairly good metabolic control (Fig 1)

*Diagnoses other than DM* Important diagnoses besides DM are listed in Table II Neuropathy retinopathy and hypertension turned out to be the most common being detected in 13–43% of the patients in groups T and I In all 22% of the patients in group T and 8% in group I suffered from angina pectoris the corresponding figures for myocardial infarction being 13 and 6% Irrespective of type of treatment 10% of the patients showed evidence of thyroid disease (mostly atoxic adenoma) Detailed data concerning this finding will be presented elsewhere

*Characteristics of patients with macroangiopathy* Altogether 52 patients in group T and 34 in group I showed clinical evidence of cerebrovascular disease ischemic heart disease (IHD) and/or intermittent claudication (macroangiopathy) These subjects were compared with pairmates of similar age and if possible of the same sex selected among patients without macroangiopathy Remaining patients also without detectable macroangiopathy were designed as non age matched controls (Table III) Age matched and not age matched group T patients with and without evidence of macroangiopathy did not differ with regard to duration of DM blood and urinary glucose serum cholesterol RBW or prevalence of hypertension However the triglyceride level in the patients with macroangiopathy (3.0±0.3 mmol/l) was significantly high than both in the age matched (2.1±0.2 mmol/l) a not age matched controls (2.3±0.2 mmol/l) T latter were younger and less hypertensive than t patients with macroangiopathy

Compared to their age matched pairmates only small proportion of the insulin treated patients wi macroangiopathy (17 vs 44%) were in fairly go state of metabolic control defined as above on t basis of urinary excretion of glucose RBW a serum triglycerides Although the differences we not statistically significant the latter two variabl tended to be less pronounced and hypertensi tended to be less common in patients without th with macroangiopathy The not age matched p tients without macroangiopathy were younge weighed less and differed from the subjects wi macroangiopathy with regard to serum trigly erides prevalence of hypertension and in th percentage of subjects considered to be in fairl good metabolic control

Data concerning smoking habits were availabl for patients with IHD Those who smoked cigarettes per day or more before 1970 or at least cigarettes per day after 1970 were classified a smokers In group T the frequency of smokers w 10/40 and 6/40 among patients with and witho evidence of IHD respectively In group I th frequency of smokers was 5/21 whether the patient showed signs of IHD or not

Table IV *Principal causes of death (%) in 140 diabetic patients treated at the Out Patient Department compared with causes of death in the general population in 1975*

| Causes of death | Diabetics | | | General population* | |
|---|---|---|---|---|---|
| | Group D ($n=10$) | Group T ($n=63$) | Group I ($n=67$) | Age group 65–74 ($n=22279$) | Age group ≥75 ($n=45775$) |
| Myocardial infarction (410) | 30 | 46 | 36 | 22 | 16 |
| Chronic IHD (412) | 0 | 16 | 18 | 15 | 24 |
| Cerebrovascular disease (430–38) | 30 | 11 | 9 | 11 | 14 |
| *Malignant neoplasms (140–209)* | *0* | 6 | *8* | 27 | 17 |
| Uremia (580–84) | 0 | 0 | 9 | 0 | 0 |
| Chronic pyelonephritis (590 0) | 0 | 0 | 5 | 0 | 0 |

International Statistical Classification of Diseases Injuries and Causes of Death (ICD 8th revision) detailed list (WHO Geneva 1967)
* Source Causes of death 1975 National Bureau of Statistics Stockholm Sweden

*Deceased patients*

RBW blood glucose and serum lipid values were mostly within the range recorded for the living patients (Table I)

Irrespective of type of therapy most deaths were due to cardiovascular disease (Table IV) Other causes of death among group I patients were uremia (9%) and infection (9%) Including the causes of death the percentage prevalence of vascular complications was higher among the deceased than the living patients This was particularly true for myocardial infarction and cerebrovascular disease (Table II) Retinopathy neuropathy and hypertension also appeared to have been more common among the deceased than living patients (Table II)

## DISCUSSION

The patients participating in the current investigation were not fully representative for all diabetics referred to the hospital as less complicated cases are seen by district physicians However there are still reasons to believe that the results do portray the efficacy of routine care in a fairly large population of diabetic patients Although in line with earlier reports (6 14 17 18 24) the outcome of our study was less encouraging as only a small proportion of the patients were in fairly good metabolic state of control Furthermore a high percentage suffered from various forms of angiopathy also evidenced by a supranormal death rate in cardiovascular disease

The effect of treatment on the development of vascular complications has been the subject of much controversy reflecting the incompleteness of our knowledge of these matters Thus it is still not known whether the angiopathy is a direct consequence of a deranged glucose metabolism rather than independent manifestations of other aquired or hereditary defects possibly aggravated by DM If the latter applies such defects as may differ between different types of DM have to be identified and treated accordingly

Several lines of evidence indicate that the mechanisms behind the development of micro- and macroangiopathy differ with impaired glucose metabolism as a common denominator The cause of microangiopathy may be unpredictable and to a variable extent unrelated to insulin deficiency (21) The models proposed for this type of vascular disease cover numerous factors including an augmented platelet aggregation (9) hyperviscosity (2) and enhanced blood level of glycosylated hemoglobins (7) i e factors that may interfere with micro circulation and oxygen transport at cellular level Although it seems to be generally agreed that microangiopathy increases with the duration of DM (17) reports conflict on the possibility of inhibiting microangiopathy by controlling blood glucose levels (4 21)

Prospective studies indicate that also macroangiopathy (atherosclerotic lesions) is of multifactorial origin In keeping with other observations (12) the current patients with and without detectable atherosclerosis could not be distinguished by the degree of hyperglycemia and glucosuria However they differed in serum triglyceride levels which were highest in those with macroangiopathy As

cholesterol concentration in general was within normal limits the enhanced triglyceride concentrations may reflect elevation of VLDL (very low density lipoproteins) Such changes in the lipoprotein pattern are often accompanied by reduction of HDL (high density lipoprotein) cholesterol (20) Whereas the association between VLDL levels and the development of atherosclerosis may be weak reduction of HDL cholesterol is recognized as a prominent risk factor (8)

The atherosclerotic group I patients differed from their age matched partners with regard to degree of metabolic control defined as above on the basis of urine glucose RBW and serum triglyceride They also tended to be more hypertensive These findings are of the utmost interest since the major risk factors (hypertension hyperlipoproteinemia smoking) detected in a general population (10) are equally important in patients with DM (11)

The current report as well as the previous papers cited above indicate that the conventional therapy for DM of mainly adult onset type is not particularly effective in delaying the development of angiopathy The data from prospective studies indirectly supported by this investigation strongly indicate the urgent need for therapeutic regimens directed not only against hyperglycemia but also against the well known risk factors mentioned above It has also been suggested that insulin contributes to the development of atherosclerotic vascular disease by stimulating arterial smooth muscle proliferation and lipid synthesis in the arterial wall (23) This observation is a further indication of the necessity of keeping body weight within normal or subnormal limits as weight reduction is known to be associated with a drop in hypertension hypertriglyceridemia and hyperinsulinism (15) Hyperinsulinism may also decrease as an effect of physical training (3) Correction of these various defects is far from easy and requires intensive co operation by the patients who have to modify their way of life more or less drastically However as conventional therapy of DM has (with some exceptions) made no major progress in recent decades we are forced to pay attention to this intricate problem which concerns a substantial part of the population

## REFERENCES


1 Albrink M J Lavietes P H & Man E B Vascular disease and serum lipids in diabetes mellitus Observations over thirty years (1931–1961) Ann Intern Med 58 305 1963
2 Barnes A J Locke P Scudder P R Dormandy T L Dormandy J A & Slack J Is hyperviscosity a treatable component of diabetic microcirculatory disease? Lancet 2 789 1977
3 Björntorp P Fahlén M Grimby G Gustafson A Holm J Renstrom P & Scherstén T Carbohydrate and lipid metabolism in middle aged physically well trained men Metabolism 21 1037 1972
4 Cahill G F Etzwiler D D & Freinkel N Control and diabetes N Engl J Med 294 1004 1976
5 Carlson L A & Bottiger L E Ischaemic heart disease in relation to fasting values of plasma triglycerides and cholesterol Lancet 1 865 1972
6 Deckert T Poulsen J E & Larsen M Prognosis of diabetics with diabetes onset before the age of thirty one II Factors influencing the prognosis Diabetologia 14 371 1978
7 Gonen B & Rubinstein A H Hemoglobin A1 and diabetes mellitus Diabetologia 15 1 1978
8 Gotto A M Status report Plasma lipids lipoproteins and coronary artery disease In Atherosclerosis review vol 4 (ed R Paoletti and A M Gotto) pp 17–28 Raven Press New York 1979
9 Heath H Bridgen W D Canever J V Pollock J Hunter P R Kelsey J & Bloom A Platelet adhesiveness and aggregation in relation to diabetic retinopathy Diabetologia 7 308 1971
10 Kannel W B Some lessons in cardiovascular epidemiology from Framingham Am J Cardiol 37 269 1976
11 Kannel W B & McGee D L Diabetes and cardiovascular risk factors The Framingham study Circulation 59 8 1979
12 Knatterud G L Klimt Ch R Levin M E Jacobson M E & Goldner M G Effects of hypoglycemic agents on vascular complications in patients with adult onset diabetes VII Mortality and selected nonfatal events with insulin treatment JAMA 240 37 1978
13 Lindberg W Natvig H Rygh Aa & Svendsen K Høyde og vektsundersøkelser hos voksne menn og kvinner Tidsskr Nor Lægeforen 76 361 1956
14 Lundbæk K Long term diabetes The clinical picture in diabetes mellitus of 15–26 years duration with a follow up of a regional series of cases Munksgaard Copenhagen 1953
15 Olefsky J Reaven G M & Farquhar J W Effects of weight reduction on obesity Studies of lipid and carbohydrate metabolism in normal and hyperlipoproteinemic subjects J Clin Invest 53 64 1974
16 Pelkonen R Nikkila E A Koskinen S Penttinen K & Sarna S Association of serum lipids and obesity with cardiovascular mortality Br Med J 2 1185 1977
17 Pirart J Diabète et complications dégénératives Présentation d une étude prospective portant sur 4400 cas observés entre 1947 et 1973 Diabète Métab 3 97 1977
18 Reinheimer W Bliffen G McCoy J Wallace D & Albrink M J Weight gain serum lipids and vas-

cular disease in diabetics Am J Clin Nutr 20 986 1967
9 Santen R J Willis P W & Fajans S S Atherosclerosis in diabetes mellitus Correlations with serum lipid levels adiposity and serum insulin level Arch Intern Med 130 833 1972
0 *Schaefer E J Anderson D W Brewer H B Jr* Levy R I Danner R N & Blackwelder W C Plasma triglycerides in regulation of H D L -cholesterol levels Lancet 2 391 1978
1 Siperstein M D Foster D W Knowles H C Jr Levine R Madison L L & Roth J Control of blood glucose and diabetic vascular disease N Engl J Med 296 1060 1977
22 Snedecor G W & Cochran W G Statistical methods 6th ed Iowa State University Press Iowa 1974
23 Stout R W Diabetes and atherosclerosis—the role of insulin *Diabetologia 16 141 1979*
24 Williams T F Martin D A Hogan M D Watkins J D & Ellis E V The clinical picture of diabetic control studied in four settings Am J Public Health 57 441 1967

Table I *Basal data on the patients participating in the study*

| Group | No of females | No of males | Age (y) | Duration of DM (y) |
|---|---|---|---|---|
| D | 6 | 9 | 53 9±3 9 | 2 4±0 6 |
| T | 77 | 47 | 66 0±1 0 | 8 6±0 5 |
| I | 102 | 76 | 55 5±1 3 | 14 6±0 7 |
| ND | 33 | 37 | 57 0±2 1 | – |

*Statistics*

Data are presented as mean ± S E M A $\chi^2$ test was used for statistical analysis of scores dealing with diets and nutrition Because of the repetition of the $\chi^2$ test the level of significance was set at $p<0\ 01$ Correlations were calculated using the Spearman correlation coefficient

## RESULTS

*Knowledge about DM (questions 1–4 11–13 T1–T2)*

Except for groups T and ND the percentage correct answers averaged more than 60 in all groups (Table II) The best results were recorded for the students (93 9±1 1%) 93% of whom scored ≥60% This level was also reached by 64% of the nursing personnel 66% of group D 52% of group T 75% of group I and 19% of group ND patients The percentage correct answers was approximately the same for all questions However more than 50% of the patients in group T did not know the basal effects of sulphonylurea treatment

*Knowledge about diets and nutrition (questions 5–18)*

The percentage correct answers in all groups tended to be lower than for the questions about DM The students score averaged 90% and that of the nursing personnel 74% This result does not differ from that of group I The non-diabetic patients scored 50% which is below the scores of groups D and T (Table II) Reasonably good scores (≥60%) were observed for only 29% of the patients in group I The corresponding figures for group T (9%) and group ND (4%) were very similar The level of knowledge among the nursing personnel was inadequate as 21% scored less than 60% The 60% level was reached by only 26% compared to 84% of the students

In general the results for individual questions varied uniformly among the groups of subjects (Table III) It is remarkable that less than 50% of th diabetic patients and only 74% of the nursing pe sonnel identified the very simple definition of c bohydrates in question 5 Similar poor results wei obtained for question 7 in which the participan had to give a good reason why diabetics are recor mended to keep to a low fat diet Only about 40 of the diabetic patients and 51% of the nursi personnel knew that fat gives rise to more ener than protein and carbohydrates The results al show that even insulin treated patients had po knowledge about such an essential nutrient as mi (question 14) and how to plan an adequate breakfa (question 17) It is still more surprising that this al applies to about 50% of the nursing personnel T best scores were obtained for questions 13 15 a 18 Here the patients had to choose between v ious alternatives for a snack (no 13) to know th juice beer and lemonade contain sugar (no 15) a that the Swedish term light milk stands for mi with a lower fat content than standard milk (no 18

*Correlations*

Increasing age in groups T and I was associat with a slight but significant decline in knowled concerning both DM and diet–nutrition (Fig 1 ar Table IV) The level of knowledge about these su jects was much the same in both sexes It showe no correlation with the duration of the patient DM the number of visits to the Out Patient D partment or whether they had been treated at t Day Care Unit However motivated patients– those claiming that they planned their meals wi the aid of dietary prescription lists tested the urine for sugar and ketones and exercised regula ly—reached better scores in diet–nutrition than th others (Table IV) Among the insulin dependent p tients significant inverse correlations were foun between test scores and relative body weight an blood glucose and serum triglyceride concentra tions

## DISCUSSION

The selection of patients for this study has bee discussed in our adjacent paper (6) We hav reasons to believe that the results portray the lev of knowledge among diabetic patients in a fairly large Stockholm area The questions offered in the test deal with simple problems of the type which

Table II *P rce t ec rrect s ers e cl gr p f part c pa ts*

The mul ple comparison $\chi^2$ test was used to compare scores dealing w h d ets and nutrit on using $p<0$ 01 as the limit of cance

| Ob ect of ques on | Group D | Group T | Group I | Group ND | Nurs ng personnel | Med cal students |
|---|---|---|---|---|---|---|
| DM as a d ease | 86 7 5 4 | 73 ? 1 | 85 9 1 4 | 63 1 3 3 | 87 1+1 8 | 93 9 1 1 |
| D et and nu on | 64 3 6 0 | 60 4+1 6 | 0 0 1 3 | 49 7 1* | 73 6+ 3 | 91 3 1 6 |

* Sco e s gn fcan ly lower than that of all o her groups except groups D and ND
* Sco e s gn fcantly lower than that of all o her groups
Sco e s gn fcantly h gher than that of a l o her groups except nurs ng personnel

d abet c pat ents have to face every day As most pat ents are probably unacqua nted w th the mult ple cho ce test method the 80% score level was chosen a b trarily as the l m t for dent fy ng the reasonably well educated pat ents

In keep ng w th previous find ngs n a much smaller ser es of pat ents from another Stockholm hosp tal (?) the current pat ents turned out to be unfam l ar w th many bas c concepts concern ng DM and the management requ red for its proper control As a general f nd ng the pat ents appeared to know more about DM than about d et and nutri t on The best results were scored by the nsul n dependent pat ents However as only 9% of these pat ents passed the 80% score l m t in the quest ons deal ng with d et–nutrition the present study clear ly demonstrates that most pat ents may be unable to treat themselves properly at home Moreover a cons derable proport on of the nurs ng personnel ev dently does not possess a bas c understand ng of d abet c d etary princ ples

Judging from the record ngs of fast ng blood glu cose urinary glucose body we ght and plasma l p ds only about 30% of the d abet cs part c pating in this study appeared to be under fa rly good metabol c control (6) Very s m lar f gures under l n ng the d ff cult es n ma nta n ng proper treat ment of non hosp talized d abetic pat ents have been reported by others (7 1?) American stud es des gned to character ze how much d abet cs know

Table III *Per ce t of p rt c p ts n eacl gro p s ng correct ans ers to nd d l q est ons*

| Ques on no | G oup D | G oup T | Group I | Group ND | Nurs ng personnel | Med cal students |
|---|---|---|---|---|---|---|
| 1 | 87 | 8 | 90 | 70 | 91 | 100 |
| 2 | 73 | 75 | 90 | 69 | 9 | 100 |
| 3 | 87 | 69 | 79 | 54 | 89 | 100 |
| 4 | 100 | 81 | 93 | 57 | 9 | 98 |
| 5 | 40 | 45 | 54 | 39 | 74 | 98 |
| 6 | 80 | 0 | 74 | 63 | 87 | 100 |
| 7 | 40 | 50 | 55 | 46 | 64 | 81 |
| 8 | 60 | 37 | 41 | 37 | 51 | 93 |
| 9 | 73 | 59 | 73 | 40 | 85 | 98 |
| 10 | 80 | 71 | 83 | 53 | 81 | 100 |
| 11 | 73 | 55 | 6_ | 49 | 60 | 77 |
| 12 | 47 | 66 | 61 | 50 | 53 | 74 |
| 13 | 87 | 86 | 94 | 54 | 9 | 91 |
| 14 | 67 | 41 | 5 | 53 | 58 | 95 |
| 15 | 80 | 79 | 89 | 66 | 94 | 100 |
| 16 | 67 | 6 | 86 | 50 | 85 | 95 |
| 17 | 47 | 41 | 64 | 4 | 49 | 7 |
| 18 | 87 | 83 | 9 | 86 | 91 | 100 |
| 1 | | | 65 | | 77 | 98 |
| 2 | | | 90 | | 44 | 95 |
| 13 | | | 97 | | 85 | 91 |
| T1 | | 46 | | | 0 | 98 |
| T? | | 86 | | | 85 | 63 |

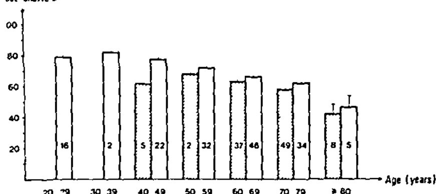



*Fig 1* Percentage c rrect answers to que ons about d et and nutr t on n d ffe e age groups □ = Group I ■ g oup T Number of pat en g en n each bar

about current concepts concern ng diabetes and the pr nc ples of treatment conclude that the level of kno vledge is too low to perm t good self control (1 9–12) Etzw ler (3) tested nurses and d etic ans and concluded that the l ck of understand ng of funda mentals of d abetes among these categor es of per sonnel may contribute to nadequate or ineffective pat ent educat on The d scourag ng results of American med cal students concern ng their knowl edge of nutr t on (8) ind cate that it would be ad vantageous to pay more attent on to this subject in the educat on program Although t is generally ac cepted that d abet c pat ents need a f rm under stand ng of the r d sease in order to manage suc cessfully at home the effect of educat on has been debated W ll ams et l (12) est mated pat ents kno ledge w th quest onna res The knowledge level wa low n general Interest ngly t correlated nve sely w th the pat ents level of control Etzwiler (4) reported that a week long intensive course in d abetes self managen ent mproved t patients knowledge level (tested by que t o na res) Th s effect was not accomp n ed by a cc respond ng change in metabolic control S milar r sults have been observed by Graber et l (5)

In summary the comb ned ev dence from seve reports clearly ind cates that management of di betes is far from adequate at present Physicia a m ng at better results face several problems cluding those regard ng cl nical soc al psycholo cal and econom c parameters However a reaso able start ng po nt should be to in prove the pat en knowledge about bas c concepts concern ng d betes and its therapy The mportance of improv knowledge is underlined by the correl t on betwe test scores n d et nutr t on and some variables diabet c control The present study demonstrat that our system w th p ecemeal instruct on is nef c ent In an attempt to enhance the patients und stand ng concern ng these matters v e have rece

Table IV C el t s bet ll e k l k e le el J d se e d t d tr t t l r s f ar te
Spearman rank correla on coeff c en

| | Group T (n 124) | | Group I (n=178) | |
|---|---|---|---|---|
| | Knowledge of DM | Knowledge of d et nutrit on | Knowledge of DM | Knowledge of d et nutr t on |
| Age of pat ents | 0 2767 * | 0 3513*** | 0 4185*** | 0 4609 ** |
| Durat on of d sease | +0 0361 | 0 0 60 | +0 1199 | 0 0190 |
| RBW | 0 06 1 | +0 1155 | 0 2 24** | 0 2252*** |
| Blood glucose | +0 0195 | 0 0371 | 0 1382 | 0 1567* |
| Urinary glucose | +0 12 8 | +0 0640 | +0 10 7 | 0 0071 |
| Serum choleste ol | 0 1431 | 0 0 77 | 0 1346 | 0 1065 |
| Serum triglycerides | 0 0162 | 0 0158 | 0 2529** | 0 2511** |
| Mot vat on | +0 0957 | +0 1980* | +0 0557 | +0 2608*** |

*p<0 05 **p<0 01 ***p<0 001

ly started a formal educational program integrated into the patient care system The results of these efforts will be the subject of future publications

## APPENDIX

*The questionnaire*

1 *Which of the following alternatives is correct?* Patients with DM have (*a*) too much sugar in blood and urine (*b*) too little sugar in blood as the sugar is excreted in the urine (*c*) no sugar in blood but plenty of sugar in the urine (*d*) no sugar in blood or urine Correct answer (*a*)

2 *What is the cause of DM?* (*a*) All organs are normal but the patient eats too much sugar The pancreas produces (*b*) too much insulin (*c*) too little insulin (*d*) The stomach produces too little gastric fluid Correct answer (c)

3 *What is the reason for the increased production of urine in patients with DM?* (*a*) Sweat production is subnormal (*b*) The patient excretes sugar in the urine which forces the kidneys to produce more urine (c) The patient is nervous and uses water as a sedative (*d*) All patients with DM suffer from kidney disease associated with a high production of urine Correct answer (*b*)

4 *The patient with DM has to test the urine regularly with Clinitest to get information about* (*a*) the amount of salts in the urine (*b*) the amount of sugar in the urine (*c*) whether the urine is infected or not (*d*) whether kidney disease is present or not Correct answer (*b*)

5 *What is carbohydrate?* (*a*) All kinds of fat (*b*) All types of nutrients that give rise to energy (*c*) A type of nutrients that contain both sugar and fat (*d*) Sugar and nutrients that can be transformed into sugar in the body Correct answer (*d*)

6 *Which of the following nutrients contains significant amounts of protein?* (*a*) Oil (*b*) syrup (*c*) apple (*d*) cod fish Correct answer (*d*)

7 *Which of the following statements is correct?* (*a*) The patient with DM is recommended to eat plenty of fat which contains valuable amounts of energy but no sugar (*b*) It is unimportant whether the patient eats small or large amounts of fat (*c*) The patient is recommended to avoid fat rich diets (*d*) The patient should eat no fat at all Correct answer (*c*)

8 *Which of the following nutrients gives rise to the largest amounts of energy?* (*a*) Protein (*b*) fat (*c*) carbohydrate (*d*) all these nutrients contain the same amounts of energy Correct answer (*b*)

9 *A patient with DM is recommended to plan his meals in the following way* (*a*) He is not permitted to eat more than 3 meals per day (*b*) He has to eat one heavy meal in the morning and one in the evening and only a light meal during the day (*c*) He ought to have breakfast lunch and dinner as well as 2–3 light meals in between (*d*) It is not necessary to plan the meals according to a special system Correct answer (*c*)

10 *Which of the following nutrients is so poor in energy content that it can be eaten without restriction* (*a*) Sugar free cakes (*b*) low fat milk (*c*) carrots (*d*) cucumber Correct answer (*d*)

11 *A glass of light beer (2% alcohol) contains the same amounts of sugar as a glass of* (*a*) sugar free lemonade (*b*) light milk (*c*) mineral water (*d*) orange juice Correct answer (*b*)

12 *How many dl of orange juice contain the same amount of energy as one fruit portion on the prescription list?* (*a*) 1 (*b*) 2 (*c*) 3 (*d*) 4 Correct answer (*a*)

13 *Which of the following dishes can be recommended as a snack?* (*a*) A hot dog (*b*) coffee or tea with an unsweetened bun and a small apple (*c*) fruit cream with 1 dl low fat milk (*d*) a cake of sugar free chocolate (diabetes type) Correct answer (*b*)

14 *A patient with DM may be permitted to drink up to 4 dl low fat milk per day Which of the following statements is correct?* (*a*) The patient has to drink the main part in the morning when the stomach is empty (*b*) It does not matter when or how the patient drinks these 4 dl milk (*c*) The patient is permitted to drink only one glass of milk at a time and the recommended interval between each glass is several hours (*d*) The patient is permitted to drink milk only together with lunch and dinner Correct answer (*c*)

15 *Which of the following statements is correct?* A patient with DM is permitted to drink unlimited amounts of (*a*) juice (*b*) light beer (*c*) Pommac (a lemonade) (*d*) none of these Correct answer (*d*)

16 *Which of the following alternatives contains too much carbohydrate to be recommendable as a light meal for a patient with DM?* (*a*) A small apple (*b*) a big banana (*c*) a slice of crisp rye bread with low fat margarine (*d*) a slice of white bread with low fat cheese Correct answer (*b*)

17 *Which of the following breakfast meals is most inappropriate for a patient with DM?* Coffee or tea (*a*) + 2 glasses of juice + 2 slices of white bread + unsweetened marmalade (*b*) + 1 glass juice + 2 slices of white bread with low fat cheese (*c*)+1 glass light milk+2 slices of white bread with smoked meat (*d*)+1 glass light milk+1 egg + a slice of white bread with brawn Correct answer (*a*)

18 *A patient with DM is recommended to use light milk instead of regular milk since light milk contains* (*a*) less sugar (*b*) more protein (*c*) more calcium (*d*) less fat Correct answer (*d*)

I1 *Acid (= ketones) in the urine means* (*a*) Urine contains blood (*b*) Sugar metabolism is poorly controlled (*c*) Urine contains bacteria (*d*) Urine is normal Correct answer (*b*)

I2 *Insulin ought to be injected subcutaneously at* (*a*) the abdomen (*b*) the outside of the upper arm (*c*) the outside of the thigh (*d*) anywhere Correct answer (*c*)

I3 *When is the most convenient time to inject the morning dose of insulin?* (*a*) Half an hour before breakfast (*b*) during breakfast (*c*) directly after breakfast (*d*) half an hour after breakfast Correct answer (*a*)

T1 *The purpose of giving sulfonylurea tablets to adult patients with DM is* (*a*) to administer insulin in tablets (*b*) to administer a compound that stimulates the pancreas to produce more insulin (*c*) to eliminate the need for dietary restrictions (*d*) to administer a compound that reduces the blood level by stimulating a high excretion of sugar in the urine Correct answer (*b*)

T2 *When is a patient with DM recommended to take*

*is tablets? (a)* Not later than one hour before breakfast *(b)* when having breakfast *(c)* half an hour after breakfast *(d)* one hour after breakfast Correct answer *(b)*

## REFERENCES


1. Beaser S B Teaching the diabetic patient Diabetes 5 146 1956
2. Burman P & Hellstrom K Ambulant kostbehandling av diabetiker Chimar eller realitet? Lakartidningen 68 4379 1971
3. Etzwiler D D Who s teaching the diabetic? Diabetes 16 111 1967
4. — The Minnesota experience Presented at the session on Evaluation of Diabetes Education Programs 20th Annual Postgraduate Course in Diabetes Jan 21 1973
5. Graber A L Christman B G Alogna M T & Davidson J K Evaluation of diabetes patient-education programs Diabetes 26 61 1977
6. Karlander S G Ålinder I & Hellstrom K Metabolic control of diabetes mellitus during routine management at an out patient department Acta Med Scand 207 475 1980
7. Lundbæk K Long term diabetes The clinical picture in diabetes mellitus of 15–25 years duration with a follow up of a regional series of cases Munksgaard Copenhagen 1953
8. Phillips M G The nutrition knowledge of medical students J Med Educ 46 86 1971
9. Stone D B A study of the incidence and causes of poor control in patients with diabetes mellitus Am J Med Sci 241 436 1961
10. Watkins J D Williams T F Martin D A Hogan M D & Anderson E A study of diabetic patients at home Am J Public Health 57 452 1967
11. Williams T F Anderson E Watkins J D & Coyle V Dietary errors made at home by patients with diabetes J Am Diet Assoc 51 19 1967
12. Williams T F Martin D A Hogan M D Watkins J D & Ellis E V The clinical picture of diabetic control studied in four settings Am J Public Health 57 441 1967

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# Hyperaminoaciduria in Mild Phosphate Diabetes in Adults

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**ABSTRACT** Quantitative urinary amino acid excretion has been studied in 23 adult patients with mild phosphate diabetes (MPD), in 22 adult control patients with various renal disorders and in 15 children 7–19 years old, with atopic disorders (normal controls) Statistically significant increases in urinary excretion of glutamine ($p<0.01$), glycine ($p<0.01$) and cystine ($p<0.05$) were found in the MPD patients compared to the normal controls The urinary excretion of glutamine was significantly increased while the increases in cystine and glycine excretions were non significant when MPD patients were compared to the control patients In addition to clinical signs and analyses of plasma and urinary phosphate a quantitative evaluation of urinary amino acids might be a tool in the diagnosis of MPD The significance of the increased urinary amino acid excretion in MPD is discussed

*Key words* mild phosphate diabetes aminoaciduria glutamine

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Phosphate diabetes is defined as hyperphosphaturia in spite of hypophosphataemia with a normal urinary calcium concentration and normal serum concentrations of calcium and parathyroid hormone Phosphate diabetes has been diagnosed considerably more often in children than in adults Falkson and Frame (5) have summarized 150 cases of mild phosphate diabetes reported in the literature Only 8% of these cases were adults Adult phosphate diabetes has also been described by Nagant and Krane (8) Phosphate diabetes gives rise to osteomalacia in children (5)

Mild phosphate diabetes in adults causes rather vague clinical symptoms such as pain in the muscles joints and bones tiredness dizziness numbness burning sensations and tenderness in the muscles and bones (7) The most common findings encountered in the laboratory besides hypophosphataemia and hypophosphaturia were high pH in the urine hyperaminoaciduria and phosphate crystals in dried urine

Mild phosphate diabetes is probably a rather common disorder but reports on its frequency are sparse in the literature The diagnosis is associated with difficulties In addition to hypophosphataemia and hyperphosphaturia with vague clinical symptoms we have observed hyperglutaminuria hypercystinuria and hyperglycinuria These observations are presented here

## METHODS

Serum phosphate was estimated according to the ACU Chem method a modification of the Certific Chem method (3) A serum concentration of phosphate ≤0 8 mmol/l was required for the diagnosis of hypophosphataemia and for the diagnosis of hyperphosphaturia a urinary excretion of at least 25 mmol/24 hours or a phosphate clearance in excess of 17 ml/min/1 73 m BSA Quantitative urinary amino acid analyses were performed on 24-hour urinary samples using a Beckman 120C Amino Acid Analyzer A lithium buffer system was used (1)

Statistical analyses of hyperaminoaciduria were performed using Wilcoxon s test

## SUBJECTS

Twenty three patients with mild phosphate diabetes earlier published by Lundberg et al (7) were studied There were 13 men and 10 women average age 49 years (range 36–64) Their mean serum phosphate concentration was 0 7 mmol/l (range 0 6–0 8) Mean phosphate clearance was 31 ml/min/1 73 m² (range 18–41)

Quantitative urinary amino acid analyses were also performed as a control in 22 adult patients 10 men and 12 women average age 43 years (range 26–60) with various renal disorders such as tubular defects nephrocalcinosis and renal calculi but without hypophosphataemia and in 15 children 7–19 years of age with atopic disorders

## RESULTS

As indicated in Tables I and II an increased urinary excretion of glutamine cystine and glycine was

Table I *Quantitative urinary excretion of glutamine cystine and glycine (µmol/24 h) in patients with phosphate diabetes patients with various renal disorders and healthy controls (mean ± S D)*

| | Glutamine | Cystine | Glycine |
|---|---|---|---|
| Mild phosphate diabetes patients (n=23) | 950±517 | 168±121 | 1 727±922 |
| Control patients (n=22) | 495±375 | 130±65 | 1 534±995 |
| Normal controls (n=15) | 493±228 | 95±74 | 933±672 |
| References (9) (n=9) | 489±215 | 75±62 | 1 687±980 |

Table II *Statistical significance of differences in urinary excretion of glutamine glycine and cyst (µmol/24 h) between the three groups analysed by Wilcoxon's test*

| | Glutamine | Cystine | Glycine |
|---|---|---|---|
| Mild phosphate diabetes/normal controls | $p<0.01$ | $p<0.05$ | $p<0.01$ |
| Mild phosphate diabetes/control patients | $p<0.01$ | n s | n s |
| Normal controls/control patients | n s | n s | $p<0.10$ |

found in patients with mild phosphate diabetes compared with control patients and healthy controls When analysed by Wilcoxon's test the urinary excretions of glutamine ($p<0.01$) glycine ($p<0.01$) and cystine ($p<0.05$) were statistically significant in mild phosphate diabetes compar with normal controls The urinary excretion glutamine but not the excretions of cystine a glycine was significantly higher in mild phospha diabetes patients than in control patients ($p<0.01$

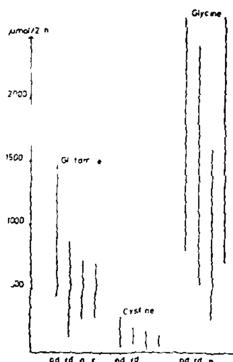



*Fig 1* Urinary excretion of glutamine cystine and glycine during 24 hours (mean + S D) *pd*=Mild phosphate diabetes *rd*=various renal disorders *n*=normal controls *r*=references

## DISCUSSION

Only a few studies of urinary amino acids in mi phosphate diabetes are reported in the literatu Dent and Harris (4) using paper chromatograph found an increase in urinary glycine excretion patients with mild phosphate diabetes resulting what they called the superglycine spot Usi quantitative amino acid analysis a far more exte sive method than paper chromatography we o served an increased urinary excretion of glutamin cystine and glycine in mild phosphate diabetes

The significantly increased urinary excretion glutamine cystine and glycine seen in mild pho phate diabetes (Tables I and II Fig 1) is hard explain It might be a specific tubular defect of t proximal part of the renal tubules in the same regi as for the reabsorption of phosphate

Pincus and Windmueller (10) have studied phosphate dependent glutaminase of the small i testine of different animals Their data indicate th the metabolism of glutamine to glutamate is d pendent upon that phosphate dependent glutami ase It is also believed that phosphate-depende glutaminase plays an important role in glutamine hydrolysis in the kidney It is also known that phos

phate is an important activator for this phosphate dependent glutaminase. Consequently a low serum phosphate concentration might diminish the activity of this phosphate-dependent glutaminase resulting in an impaired hydrolysis of glutamine in the renal tubules.

Generalized renal hyperaminoaciduria and hyperphosphaturia has been described by several authors in children with vitamin D deficient rickets (2). This is caused by a defective reabsorption of amino acids in the proximal part of the tubules. According to Fraser et al. (6) the disturbance of transport affecting the absorption of phosphate and amino acids in human vitamin D deficiency is caused by an excess of parathyroid hormone rather than a simple deficiency of vitamin D in the renal tubular epithelial cells. In mild phosphate diabetes the parathyroid hormone levels have, however, been within the normal range.

Hyperaminoaciduria is not present in all patients with mild phosphate diabetes. Analysis of urinary amino acids is nevertheless a valuable diagnostic tool when tracing this difficult disorder.

## REFERENCES


1. Benson J V, Gordon M J & Pattersson J A. Accelerated chromatographic analysis of amino acids in physiological fluids containing glutamine and asparagine. Anal Biochem 18: 228, 1967.
2. Chisolm J & Harrison H E. Aminoaciduria in vitamin D deficiency states in premature infants and older infants with rickets. J Pediatr 60: 206, 1962.
3. Daly J A & Ertingshausen G. Direct method for determining phosphate in serum with the "Centrific Chem". Clin Chem 18: 263, 1972.
4. Dent C E & Harris H. Hereditary forms of rickets and osteomalacia. J Bone Joint Surg 38B: 204, 19 6.
5. Falkson G & Frame B. Phosphate diabetes: a review. Henry Ford Hosp Med Bull 6: 244, 1958.
6. Fraser P, Kooh S W & Scriver C R. Hyperparathyroidism as the cause of hyperaminoaciduria and phosphaturia in human vitamin D deficiency. Pediatr Res 1: 425, 1967.
7. Lundberg E, Bergengren H & Lindqvist B. Mild phosphate diabetes in adults. Acta Med Scand 204: 93, 1978.
8. Nagant de Deuxchaisnes C & Krane S M. The treatment of adult phosphate diabetes and Fanconi syndrome with neutral sodium phosphate. Am J Med 43: 508, 1967.
9. Niederwiesner A & Pataki G. New techniques in amino acid, peptides and protein analysis, pp. 8–9. Ann Arbor Science Publishers, Ann Arbor 1971.
10. Pincus L M & Windmueller H G. Phosphate dependent glutamine of small intestine. Localization and role in intestinal glutamine metabolism. Arch Biochem Biophys 182: 506, 1977.

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# Noise as a Contributory Factor in the Development of Elevated Arterial Pressure

*A Study of the Mechanisms by which Noise may Raise Blood Pressure in Man*

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ABSTRACT Arterial pressure and other hemodynamic variables (stroke volume (SV), cardiac output and total peripheral resistance) were studied in 18 healthy males before and during exposure to recorded industrial noise All measurements took place under strictly standardized conditions in a noise laboratory The frequency distribution and level of noise used for stimulation were continuously monitored and kept constant within close limits throughout the experiments SV was measured with impedance cardiography Indirect blood pressure (BP) in the brachial artery was measured with an automatic device and the derived parameters, cardiac output and total peripheral resistance were calculated from these measurements Compared with resting conditions at 40 dBA stimulation with industrial noise at 95 dBA caused significant increases in diastolic BP, mean arterial pressure and total peripheral resistance Minor but statistically significant reductions of SV and cardiac output were seen Heart rate and systolic BP did not change These alterations of the hemodynamic variables persisted throughout 20 min of noise stimulation and were maintained for 5 min following cessation of noise stimulation All variables had returned to their initial levels 10 min after discontinuation of noise stimulation This study suggests that exposure to industrial noise at levels prevailing during several industrial processes may cause acute elevations of arterial BP and peripheral vascular resistance In animal studies, repeated elevations of BP due to exposure to noise have been shown to cause a permanent elevation of BP Therefore we suggest that noise may be one of several external stimuli contributing to the development of arterial hypertension in man

*Key words* hypertension noise hemodynamics

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The etiology of hypertension in man is not fully known Few would oppose the opinion that hereditary factors play an important role Great interest has also been devoted to external variables e g ingestion of salt (4) either as primary causes of hypertension or as modulating influences

Among several conceivable external factors which may affect the development of hypertension we became interested in noise after having observed that industrial workers with a severe noise induced impairment of hearing indicating prolonged and severe exposure to industrial noise had significantly higher blood pressures (BP) than subjects of the same age and sex but with normal hearing and that the rate of hypertension was higher in the group exposed to noise (8) Previous animal studies mainly in rats have shown acute rises of BP during stimulation with noise (12 19) Repeated exposure of animals to alerting stimuli has also been shown to produce a permanent elevation of BP (3 16)

This report describes the acute hemodynamic effects of exposure to industrial noise under carefully standardized conditions The purpose has been to establish whether exposure to noise at levels which frequently occur in occupational life may cause a rise of BP also in man and if so by which hemodynamic mechanism

## METHODS

Eighteen normotensive male volunteers took part in the investigation All had normal auditory acuity (subjectively

*Abbreviations* BP = blood pressure SV = stroke volume HR = heart rate

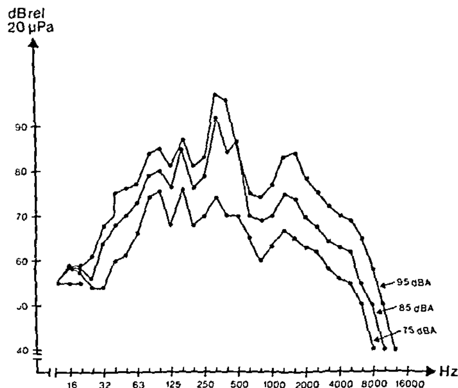



*Fig 1* Frequency nal and sound levels of the dustrial noise induced i noise laboratory

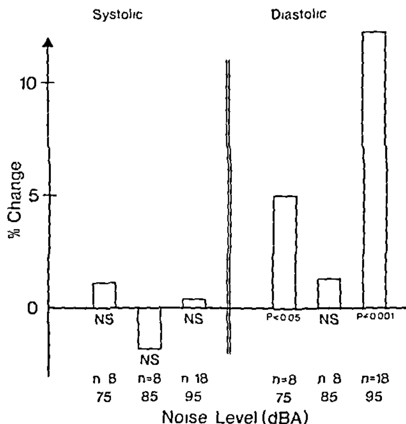



*Fig 2* Changes in systolic and diastolic BP during st mulation with 75 85 and 95 dBA for peri of 10 min compared with in tial levels following 20 min of rest a dBA

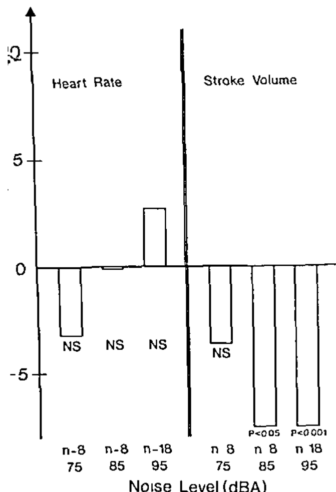



*Fig 3* Changes in HR and SV induced by noise stimulation at 75 85 and 95 dBA for periods of 10 min compared with initial values following 20 min of rest at 40 dBA

nd tested with audiometry) Their average age was 26 years (range 23-31) None of them had taken medication of any kind on the day of investigation Their average recumbent BP after 20 min rest was 120/70 mmHg

The hemodynamic variables were studied with non invasive methods Systolic and diastolic BPs were measured indirectly in the brachial artery using an automatic BP recorder (Bosch Bosomat) The accuracy of this equipment had been assessed by simultaneous comparisons with BP measurements with a mercury sphygmomanometer The correlation coefficient between simultaneous measurements was $r=0\ 93$ $p<0\ 001$

Stroke volume (SV) was measured with impedance cardiography (IFM Minnesota 304 A) in the recumbent position Two conductive Mylar strip electrodes (Electrode Tape No M 6001 3M Co) were placed around the neck and separated as widely as possible A third electrode was placed around the thorax at the level of the xiphoid process and a fourth around the upper abdomen not less than 3 cm from the third electrode The output signals of the cardiograph were recorded on a Siemens Elema Recorder (34 T) SV was calculated according to the $\Delta Z$ formula (5)

$$SV \quad A \quad L \quad \frac{\Delta Z}{Z_0}$$

where $A$ is the cross section area of the thorax at the level of the third electrode $L$ the distance between the second and the third electrode (average of ventral and dorsal measurements) $\Delta Z$ the maximal amplitude of the waveformed impedance change during systole and $Z_0$ the basic impedance between the second and the third electrode

This method correlates very well with other ways of determining SV and cardiac output In our laboratory we compared this method with the dye-dilution method using a densitometer (Cardiognost Atlas) and indocyanine green (Cardio-Green) in 13 paired experiments The mean value of cardiac output determined by the impedance cardiography method did not differ significantly from that obtained by the dye-dilution method (8 39 l/min vs 7 49 l/min $t$ 1 86 n s ) A statistically significant correlation was found between measurements obtained by the two methods ($r=0\ 78$ $p<0\ 002$)

All experiments were performed in a specially equipped noise laboratory The subjects rested comfortably in the

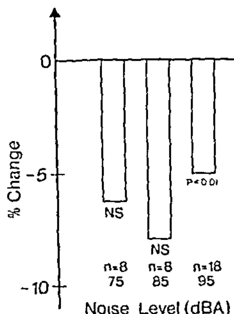



*Fig 4* Changes in cardiac output induced by noise stimulation at 75 85 and 95 dBA for periods of 10 min compared with initial values following 20 min of rest at 40 dBA

recumbent position on a couch in the center of an exposure room with eight loudspeakers built into the walls Tape recorders amplifiers and equipment for acoustical measurements were placed in an adjacent control room Recorded industrial noise was replayed through the loudspeakers The noise level in the exposure room was measured continuously throughout the studies at the ear level approximately 10 cm from the subject s head The A weighted noise level and the frequency spectrum of the noise were analyzed (Fig 1) This made it possible to maintain a constant noise level (±1 dBA) over the entire frequency spectrum throughout the experiments The equipment for acoustical measurements was manufactured by *Bruel & Kjaer (microphone type 4145* preamplifier type 2619 *real* time analyzer type 3347 sound level recorder type 2305 sound level analyzer type 4426) Measurements were made after 20 min of recumbent rest at 40 dBA and again after exposure to noise at 95 dBA for 20 min In eight subjects recordings were made also after stimulation for 10 min at 75 and 85 dBA Finally recordings were made at 5 10 and 15 min after cessation of noise stimulation

Standard methods were used for calculation of the mean S D and *r* value The hypothesis of no difference in means was tested by the *t* test for paired data. Only two-tailed tests were used and differences were considered significant for *p* values <0 05

## RESULTS

Following 20 min of recumbent rest at 40 dBA the average systolic BP was 120±2 9 mmHg diastolic BP 70±2 0 mmHg mean arterial pressure 87±2 0 mmHg heart rate (HR) 61±2 5 beats/min SV 113±5 6 ml cardiac output 7 0±0 5 l/min total peripheral resistance 13 7±1 3 PR units During stimulation with noise at 95 dBA a statistically significant increase in diastolic BP but no change in systolic BP occurred (Fig 2)

No significant changes in HR occurred during stimulation with noise at either 75 85 or 95 dBA whereas significant reductions of SV were seen at the two highest levels of stimulation (Fig 3) The reduction of SV also caused a significant reduction of cardiac output at the highest level of stimulation (Fig 4) Statistically significant increments of mean arterial pressure and total peripheral resistance occurred during stimulation with noise at the 95 dBA level (Fig 5)

The observed hemodynamic changes remained virtually unaltered 5 min after cessation of the noise stimulus All hemodynamic variables had however returned to their initial levels 10 min after discontinuation of noise stimulation

## DISCUSSION

Short lasting stimulation with industrial noise at 95 dBA under carefully standardized conditions in a noise laboratory caused a statistically significant increase in diastolic BP in 18 healthy volunteers but no change in systolic BP This agrees with findings by others during stimulation with aircraft and traffic noise (13 14) The increase in diastolic BP could be ascribed to a significant rise of total peripheral resistance A noise induced increase in diastolic BP concomitant with vasoconstriction in peripheral vascular beds has previously been reported (11) This pattern of hemodynamic responses resembles in part those seen during the defense reaction (1) The lack of cardiac participation in the rise of BP (no increase in HR or SV) is reflected by the unchanged systolic BP This is most likely caused by reflex inhibitory effects on the heart through the baroreceptor mechanism Thus the suppression of the baroreceptor reflex seen during more fulminant forms of the defense reaction (7) does not seem to take place during stimulation with noise at the sound levels employed in the present study

Previous studies in animals have shown marked acute elevation of BP in some species during stimulation with noise (12 16 19) Noise alone and com

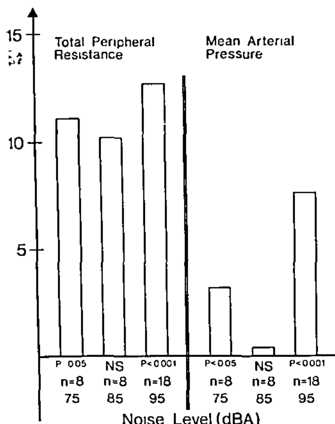



*Fig 5* Changes in total peripheral resistance and mean arterial BP induced by noise stimulation at 75 85 and 95 dBA for periods of 10 min compared with initial levels following 20 min of rest at 40 dBA

ied with other stress stimuli can also induce permanent hypertension in animals (17)

Evaluating the present results against this ckground it is conceivable that stimulation with ise e g in the form of industrial noise is one of veral external stimuli by which persistently eleted BP may be elicited in man In other words normotensive individuals are exposed to noise vels severe enough to cause an acute rise of BP it conceivable that repeated exposure to such imuli could cause persistent hypertension Our ndings that industrial workers with a noise induced impairment of hearing had higher BPs and a igher incidence of hypertension than individuals ith normal hearing (8) support indirectly this view his suggestion is corroborated by the fact that a evere noise induced impairment of auditory acuity equently requires almost daily exposure to noise or periods longer than 10 years (10) The view that rolonged exposure to noise may cause hypertension is further supported by the recent observation hat the prevalence of hypertension and other cardiovascular disorders was approximately 50% higher in areas exposed to aircraft noise (9) It has also been shown that the prevalence of borderline and established hypertension is significantly higher among weavers in textile mills exposed for several years to daily noise levels of more than 90 dBA (mean 96) than among workers not exposed to noise (15) On the other hand no relation between exposure to noise and hypertension has been found in some other studies (2 16)

We found no linear relationship between noise level and BP response The reason for this could be that the more abrupt change in noise from 40 to 75 dBA induced a greater shift in noise level than did the change from 75 to 85 dBA Another possible explanation could be that the initial hemodynamic changes at 75 dBA might be secondary to a combination of the exposure to noise and expectancy stress

Obviously it is not possible to draw definite conclusions concerning the importance of audiogenic stimulation in the pathogenesis of essential hyper

tension Genetic factors are likely to be of great importance in determining the susceptibility to noise stimulation This can be illustrated by studies in rats where one strain developed hypertension whereas another exposed to the same kind of noise did not (16) It is also conceivable that individuals with a genetic predisposition to develop hypertension may hyper react to stressful stimuli in analogy with observations by Hallbäck (6) who noted a more pronounced rise of BP in young prehypertensive spontaneously hypertensive rats e g during exposure to noise than in normotensive rats

Thus noise could be one of several external factors contributing to the development of hypertension in man particularly in genetically suspectible individuals The relative importance of noise in this respect is difficult to evaluate Nevertheless it is interesting that increased sensitivity to salt has been noted in animals with audiogenic hypertension (16) a finding that further illustrates the complexity of this matter

In conclusion short lasting exposure of healthy volunteers to industrial noise caused an acute rise of diastolic BP due to an increase in vascular resistance It is conceivable that repetition of such stimuli may cause a persistent elevation of BP and that noise therefore could be one of several external factors contributing to the development of hypertension

## ACKNOWLEDGEMENTS

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## REFERENCES

1 Abrahams V C Hilton S M & Zbrozyna A W The role of active muscle vasodilatation in the alerting stage of the defense reaction J Physiol 171 189 1964
2 Drettner B Hedstrand H Klockhoff I & Svedberg A Cardiovascular risk factors and hearing loss Acta Otolaryngol 79 366 1975
3 Folkow B & Rubinstein E H Cardiovascular effects of acute and chronic stimulations of the hypothalamic defense area in the rat Acta Physiol Scand 68 48 1966
4 Freis E D Salt volume and the prevention of hypertension Circulation 53 589 1976
5 Granerus G & Elg R Hjartminutvolymbestamning med impedanskardiografi Berakning av slagvolymen från den icke deriverade signalen (AZ) Lakaresallskapets Riksstamma p 157 1975
6 Hallback M Interaction between central neurogenic mechanisms and changes in cardiovascular design in primary hypertension Acta Physiol Scand (Suppl) 424 1975
7 Hilton S M Inhibition of baroreceptor reflexes on hypothalamic stimulation J Physiol 165 56 1963
8 Jonsson A & Hansson L Prolonged exposure to a stressful stimulus (noise) as a cause of raised blood pressure in man Lancet I 86 1977
9 Knipschild P Medical effects of aircraft noise Community cardiovascular survey Int Arch Occup Environ Health 40 185 1977
10 Kylin B Temporary threshold shift and auditory trauma following exposure to steady state noise Acta Otolaryngol (Suppl) 152 1 1960
11 Lehmann G & Tamm J Uber Veranderungen der Kreislaufdynamik des ruhenden Menschen unter Einwirkung von Gerauschen Int Z Angew Physiol 16 217 1956
12 Medoff H S & Bongiovanni A M Blood pressure in rats subjected to audiogenic stimulation Am J Physiol 143 300 1945
13 Mosskov J I & Ettema J H Extra auditory effects in short term exposure to aircraft and traffic noise Int Arch Occup Environ Health 40 165 1977
14 — Extra auditory effects in long term exposure to aircraft and traffic noise Int Arch Occup Environ Health 40 177 1977
15 Parvizpoor D Noise exposure and prevalence of high blood pressure among weavers in Iran J Occup Med 18 730 1976
16 Rothlin E Cerletti A & Emmenegger H Experimental psycho-neurogenic hypertension and its treatment with hydrogenated ergot alkaloids (Hydergine) Acta Med Scand (Suppl) 312 27 1956
17 Smookler H Goebel K Siegel M & Clarke E Hypertensive effects of prolonged auditory visual and motion stimulation Fed Proc 32 2105 1973
18 Takala J Varke S Vaheri E & Sievers K Noise and blood pressure Lancet 2 974 1977
19 Yeakel E H Shenkin H A Rothballer A B & McCann S McD Blood pressures of rats subjected to auditory stimulation Am J Physiol 155 118 1948

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# Thyrotoxic Hypercalcemia Treated with Corticosteroids

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ABSTRACT A 42 year old man presenting with symptomatic hypercalcemia was successfully treated with corticosteroids Initially he was thought to suffer from Addison s disease A thyrotoxic state was however disclosed during the treatment Evidence suggests that the hypercalcemia was caused by thyrotoxicosis The effects of thyroid and adrenocortical hormones on calcium metabolism are discussed Corticosteroids seem valuable in differentiating thyrotoxic hypercalcemia from coincidental hyperparathyroidism

*Key words* thyrotoxicosis hypercalcemia corticosteroids

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Symptomatic hypercalcemia is an unusual manifestation of thyrotoxicosis and may present a diagnostic problem when the usual features of thyrotoxicosis are not marked or are concealed by the hypercalcemic state The patient presented here emphasizes these difficulties

## CASE REPORT

A 42 year old man with urogenital tuberculosis 7 years ago was admitted to our department with nausea anorexia and weight loss Anxiety tremor and sweating had occurred in Feb 1977 and the patient was treated with antidepressants In May 1977 he also experienced nausea vomiting extreme thirst and polyuria

On admission on October 3 1977 he had lost 30 kg in weight and had been unable to retain food for the last few days He was dehydrated and apathetic and had a blood pressure of 160/90 mmHg and a pulse rate of 104/min His body weight was 42 7 kg height 170 cm Increased truncal pigmentation was observed The tendon reflexes were symmetrical but weak There was no enlargement of the thyroid gland or thyrotoxic eye signs Laboratory analyses showed Hb 11 2 g/dl ESR 57 mm/h The serum concentration of sodium was 133 mM chloride 88 mM potassium 4 0 mM total protein 71 g/l albumin 37 g/l and creatinine 144 $\mu$M (creatinine clearance 0 93 ml/sec) Liver function tests were slightly abnormal The serum calcium concentration was 3 75 mM serum phosphorus 0 93 mM (Fig 1) Thyroid function tests showed elevated values (Table I) and increased serum levels of thyroid microsomal antibodies Serum parathormone (PTH) (radioimmunoassay) was within the lower limit of the normal range (0 2 $\mu$g/l) ECG showed prolonged PQ time of 0 24 sec shortened QT time and precordial T wave inversions X ray examinations revealed calcium deposits in the liver and the right kidney

*Clinical course and treatment*

Initially the patient was thought to suffer from primary hyperparathyroidism with threatening hypercalcemic crisis During the first 24 hours he was treated with i v fluid and electrolytes (sodium chloride and isotonic glucose) and serum calcium fell from 3 75 to 3 30 mM (Fig 1) On the next day 40 mg prednisone was added for four consecutive days to evaluate the diagnosis of hyperparathyroidism During this treatment the serum calcium fell to 3 04 mM (Fig 1) and the urinary excretion of calcium from 14 0 to 10 3 mM/day The patient s condition improved dramatically with disappearance of nausea and anorexia However when the corticosteroid medication was withdrawn a severe relapse occurred With his earlier tuberculous manifestations in mind the increased skin pigmentation and the excellent effect of corticosteroid therapy we considered the possibility of Addison s disease with symptomatic hypercalcemia From the 11th day the patient was given cortisone acetate 25 mg twice a day and fluorhydrocortisone acetate 0 10 mg daily

The patient became gradually normocalcemic (Fig 1) and the serum creatinine normalized The general condition improved his appetite increased and in three weeks he gained 10 kg in weight The main residual symptoms were extreme thirst polyuria (7 500 ml/24 h) and isostenuria

During the corticosteroid treatment the patient developed clinical symptoms of thyrotoxicosis with tremor tachycardia and sweating PBI and $T_3$ values (Table I) on admission confirmed the diagnosis of thyrotoxicosis Control tests showed marked reduction of thyroid hormones during corticosteroid therapy (Table I) and there was no TSH response after TRH injection The results of plasma cortisol before and after Synacthen injection revealed a normal adrenocortical response The corticosteroid treatment was discontinued and replaced by carbimazole and propranolol

The patient was discharged three weeks later without thyrotoxic symptoms Serum calcium values were persistently normal and he had gained 15 kg in weight Six months later he was still euthyroid and normocalcemic and weighed 70 kg

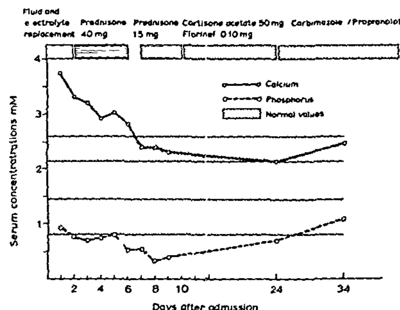



*Fig. 1* Serum concentrations of calcium and phosphorus during treatment. Daily medication is indicated on the top of the figure.

## DISCUSSION

The initial symptoms in our patient were obviously due to thyrotoxicosis but were later concealed by the symptoms of hypercalcemia. A normal PTH value and the suppression of hypercalcemia by corticosteroids exclude the possibility of hyperparathyroidism. Serum calcium remained normal during antithyroid treatment. Thyrotoxicosis was thus evidently the primary disorder.

Hypercalcemia caused by thyrotoxicosis was first reported in 1937 by Wijnbladh (18). In 1966 Baxter and Bondy (2) reported an incidence of 19–23%. Later reports have presented figures between 2 and 53% (2, 10, 15). Free ionized calcium is increased in thyrotoxic patients (9, 15) and is probably correlated to the degree of thyrotoxicosis (3). However, Adams et al. (1) found no correlation in terms of severity between hypercalcemia (total serum calcium) and thyrotoxicosis.

Table 1. *Thyroid hormone values (nM) before and after three weeks of corticosteroid treatment*
Percentage reduction in parentheses

| | Before | After | Normal range |
|---|---|---|---|
| PBI | 1 466 | 665 (55 | 315–630 |
| $T_4$ | 208 | – | 70–140 |
| $T_3$ | 3 6 | 2 8 (22 | 1 2–2 8 |

Thyrotoxic hypercalcemia is usually mild and without clinical implications. Clinical studies indicate that symptomatic hypercalcemia is associated with hyperparathyroidism in about 50% of cases (17), and patients with hyperparathyroidism show an incidence of 1–2% of associated thyrotoxicosis (11, 17). Severe illness may depress elevated T3 values in thyrotoxicosis (8). In our patient T3 was only slightly elevated, possibly due to the poor general condition. This may have been a contributory factor in masking his thyrotoxic state.

Thyroid hormones enhance bone resorption (13). The stimulating effect on the osteoclast activity is completely inhibited by calcitonin and partly by cortisol (16).

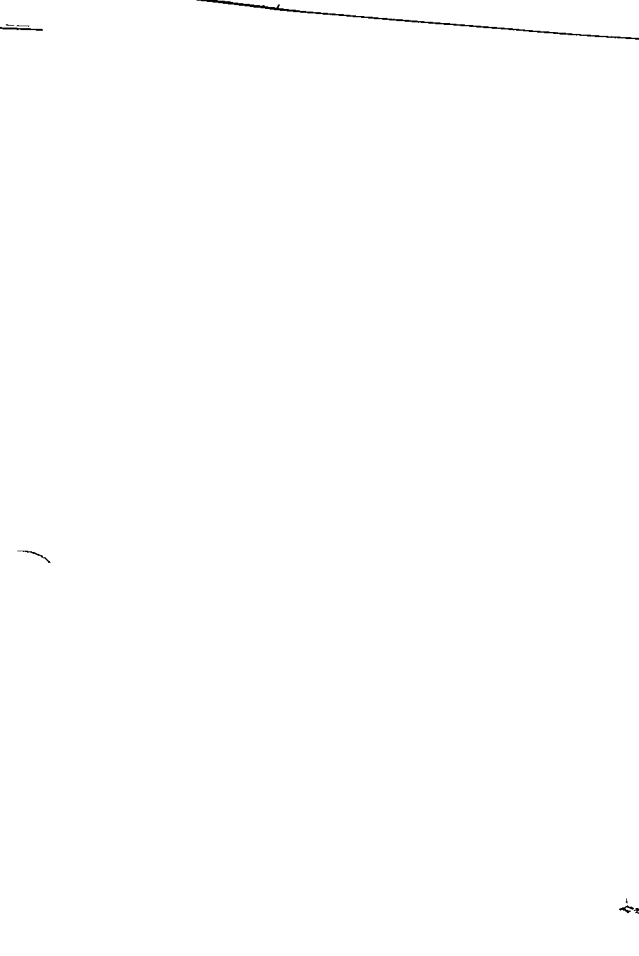
Renal excretion of calcium is increased by thyroid and adrenocortical hormones (12, 14). Polyuria in thyrotoxicosis may thus be due to combined hormonal and hypercalcemic renal effect. In our patient polyuria persisted despite normalization of serum calcium, and lasted until the thyroid function had become normal.

Corticosteroids influence the metabolism of thyroid hormones with reduced T3 (5, 6). In thyrotoxicosis, T4 is also reduced by corticosteroids indicating a direct effect on the thyroid gland (19). Calcitonin has been reported to be a valuable tool in handling thyrotoxic hypercalcemia (4, 20). However, calcitonin also has a pronounced calcium lowering effect in hyperparathyroidism. It

is therefore without value in the differentiation between hyperparathyroidism and other causes of hypercalcemia In this respect corticosteroids are a diagnostically useful tool (7) The marked effect of corticosteroids in our patient demonstrates both the therapeutic and the diagnostic value of this drug

## REFERENCES


1 Adams P Lowsey J Kelly P J Riggs B J Kinney V R & Jones J D Effects of hyperthyroidism on bone and mineral metabolism in man Q J Med 36 1 1967
2 Baxter J D & Bondy P K Hypercalcemia of thyrotoxicosis Ann Intern Med 65 429 1966
3 Bergdahl L Hyperparathyroidism in thyrotoxicosis Am J Surg 133 _06 1977
4 Buckle R M Mason A M S & Middleton J E Thyrotoxic hypercalcemia treated with porcine calcitonin Lancet 1 1128 1969
5 Burr W A Ramsden D B Griffiths R S Black E G & Hoffenberg R Effect of a single dose of dexamethasone on serum concentrations of thyroid hormones Lancet 2 58 1976
6 Chopra I J Williams D E Orgiazzi J & Solomon D H Opposite effects of dexamethasone on serum concentrations of 3 3 5 triiodothyronine (reverse T3) and 3 3 5 triiodothyronine (T3) J Clin Endocrinol Metab 41 911 1975
7 Dent C F & Watson L The hydrocortisone test in primary and tertiary hyperparathyroidism Lancet 2 662 1968
8 Engler D Donaldson E B Stockigt J R & Taft P Hyperthyroidism without triiodothyronine excess An effect of severe non thyroidal illness J Clin Endocrinol Metab 46 77 1977
9 Frizel D Malleson A & Marks V Plasma levels of ionized calcium and magnesium in thyroid disease Lancet 1 1360 1967
10 Gordon D L Suvanich S Erviti V Schwartz M A & Martinez C J The calcium level and its significance in hyperthyroidism A prospective study Am J Med Sci 268 31 1974
11 Heimann P Nilsson O & Hansson G Parathyroid and thyroid disease Thyroid disease connected with hyperparathyroidism Acta Chir Scand 135 143 1970
12 Katz A I Emmanouel D S & Lindheimer M D Thyroid hormone and the kidney Nephron 15 223 1975
13 Krane S M Brownell G L Stanbury J B & Corrigan H The effect of thyroid disease on calcium metabolism in man J Clin Invest 38 874 1956
14 Laake H The action of corticosteroids on the renal absorption of calcium Acta Endocrinol 34 60 1960
15 Mosekilde L & Christensen M S Decreased parathyroid function in hyperthyroidism Interrelationship between serum parathyroid hormone calciumphosphorus metabolism and thyroid function Acta Endocrinol 84 566 1977
16 Mundy G R Shapiro J L Bandelin J G Canalis E M & Raisz L G Direct stimulation of bone resorption by thyroid hormones J Clin Invest 58 529 1976
17 Parfitt A M & Dent C E Hyperthyroidism and hypercalcemia Q J Med 34 171 1970
18 Wynbladh H Uber die thyreotoxischen Krisen und ihre Behandlung mit besonderer Berucksichtigung der Jodbehandlung Acta Chir Scand 79 507 1936
19 Williams D E Chopra I J Orgiazzi J & Solomon D H Acute effects of corticosteroids on thyroid activity in Graves disease J Clin Endocrinol Metab 41 354 1975
20 Woodhouse N J Y Hoare A Mohamedally S M & Marsden P Thyrotoxicosis and hypercalcemia Response to antithyroid drugs and salmon calcitonin Horm Res 7 238 1976



Acta Med Scand 207 503–504 1980

# Severe Angina Pectoris and β-Blocker-Induced Bradycardia Treated with an Artificial Pacemaker

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**ABSTRACT** A 54 year old man who was treated with propranolol for severe angina pectoris developed severe symptomatic bradycardia during this treatment Coronary angiography revealed severe coronary artery stenosis, but a bypass operation was judged to be technically impossible When propranolol was withdrawn the effort angina deteriorated and anginal pains even developed at rest A favourable symptomatic effect was obtained with a combined regimen of propranolol and a permanent demand pacemaker Nitroglycerin consumption was reduced from about 20 to less than 3 tablets a day His condition remained stable during the observation period of 44 months The symptomatic effect of a β-blocking agent combined with a permanent pacemaker is considered to be due to the reduced inotropic and chronotropic effect of propranolol during exercise as well as the elimination of a bradycardia induced angina at rest Placebo effect to a certain extent cannot be excluded

*Key words* blood pressure heart rate

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Beta adrenergic blocking agents have been used in the treatment of angina pectoris for several years and their symptomatic effects have been clearly documented (3) The effect is considered to be due to a reduced heart rate (HR)/blood pressure (BP) product especially during exercise resulting in a decreased myocardial oxygen demand An additional effect is obtained in the hypertensive patient by reducing the BP both at rest and during exercise Unfortunately a severe symptomatic bradycardia may occur in some patients who otherwise obtain relief with this therapy (5) If coronary surgery can not be performed the patient has to rely on nitrates and calcium blocking agents sometimes with limited relief of symptoms

In recent years we have tried to treat a few such patients with a combination of a permanent pacemaker and a β adrenergic blocking agent We report a patient who responds favourably to this kind of treatment

## CASE REPORT

The patient a male born in 1925 had been hypertensive since 1945 and developed angina pectoris in 1971 Myocardial infarction has never been diagnosed In 1972 he was given practolol 200 mg/day resulting in a good symptomatic effect on the anginal attacks During this treatment the resting HR was reduced to 40/min In 1973 a coronary angiography revealed severe coronary artery stenoses not acceptable for a bypass operation

During the following year the patient reported increasing effort angina as well as anginal pains at night rest A bicycle ergometer test was performed during continued practolol treatment He was exercised up to 300 kpm/min reached a HR of 70/min and performed a total work of 2 100 kpm

Practolol was withdrawn from the market in 1975 and he was given propranol 240 mg/day divided into three doses The patient now reported troublesome vertigo and was shortly thereafter admitted to our department because of a syncope The HR was 35/min and ECG showed a sinus bradycardia An attempt to discontinue the propranolol treatment resulted in a marked deterioration of his angina pectoris A permanent rate programmable demand pacemaker (Omni Stanicor) was implanted The initial stimulation rate 70 ppm was reduced later to 60 ppm in keeping with the patient s preference Continuing on the initial dose of propranolol the patient reported improvement of the anginal attacks both on effort and at night The daily consumption of nitroglycerin was reduced from about 20 to less than 3 tablets He was now able to perform nearly normal physical activities The vertigo had disappeared and he did not suffer from syncopal attacks until the pacemaker suddenly failed after 34 months because of battery depletion He then suddenly experienced extreme fatigue vertigo and impairment of his angina ECG

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*Abbreviations* BP=blood pressure HR=heart rate

monitoring revealed extreme sinus bradycardia with HRs of 10–30/min. Implantation of a new pacemaker was followed by complete recovery.

At the end of the total observation period of 44 months a new bicycle ergometer test was performed during continued propranolol medication. The patient could be exercised up to 300 kpm/min, reached a HR of 70/min and performed a total work of 2 100 kpm. ECG during external overdrive suppression of the implanted pacemaker revealed a sinus bradycardia with a HR of 35–40/min. During the observation period the relative cardiac volume had increased from 550 to 800 ml/m².

One year after implanting the first pacemaker the patient reported increasing dyspnoea on exertion supposed to be caused by a left ventricular failure. When digoxin and furosemide were added to the medication, the symptoms disappeared and no further signs of cardiac failure were observed.

## DISCUSSION

The effect of $\beta$ adrenergic blocking agents on angina pectoris is considered to be due to a reduced myocardial oxygen requirement as a result of reduced HR and systolic BP, especially during exercise (1). Some bradycardia commonly occurs also in the resting state, but bradycardia related symptoms are unusual on doses required to obtain relief from angina pectoris (5). Bradyarrhythmias, however, are considered to contraindicate institution of treatment with such agents.

In our patient, withdrawal of propranolol was followed by deterioration of the effort angina and it was not possible to find a dosage that relieved the pains without inducing symptomatic bradycardia. A combined therapeutic approach, comprising propranolol medication and implantation of a permanent demand pacemaker, was therefore considered. The symptomatic effect of this treatment was impressive, with a considerable reduction of the nitroglycerin requirement. The bicycle ergometer tests before and after pacemaker implantation, both performed during adequate treatment with $\beta$ blocking agents, were almost identical. The possibility of a placebo effect due to the attention and the operative procedure cannot be completely excluded, but the persistent improvement for several years seems to rule out such an effect as the sole cause.

There are few clinical reports concerning this problem. Warren and Goldberg (6) reported excellent symptomatic effect of combined treatment with propranolol and a permanent pacemaker in three patients with angina pectoris unfit for surgery. As in our patient, however, objective criteria for the improvement were difficult to obtain. By implanting a pacemaker which can be programmed to a stimulating frequency of 60/min the negative chronotropic effect of the $\beta$ receptor blocking agent can be almost abolished. This will eliminate the bradycardia related cerebral symptoms and possibly the nightly angina when caused by profound bradycardia. Extreme bradycardia and angina at rest was observed in our patient when the first pacemaker was exhausted. On the other hand, the $\beta$ blockers also protect the myocardium by reducing the tendency to unnecessary tachycardia and BP elevation during exercise. Rivier et al. (4) pointed out that the inotropic effect may act independently of the chronotropic effect. Boudoulas et al. (2) similarly showed a differential time course of inotropic and chronotropic effect after oral propranolol. So even if the negative chronotropic effect is abolished by the pacemaker in the resting state, the myocardial oxygen requirement may still be reduced because of decreased myocardial contractility.

Treatment with $\beta$ blockers involves some risk of precipitating heart failure. Our patient developed moderate left ventricular failure and an increasing cardiac volume, but when treated with digoxin and furosemide he could continue with propranolol without further problems.

## REFERENCES


1. Amsterdam, E. A., Hughes, J. L., De Maria, A. N., Zelis, R. & Mason, D. T.: Indirect assessment of myocardial oxygen consumption in the evaluation of mechanisms and therapy of angina pectoris. Am J Cardiol 33: 737, 1974.
2. Boudoulas, H., Beaver, B. M., Kates, R. E. & Lewis, R. P.: Differential time course of inotropic and chronotropic blockade after oral propranolol. Cardiovasc Med May: 511, 1977.
3. Prichard, B. N. C.: Propranolol in the treatment of angina. A review. Postgrad Med J (Suppl) 4: 35, 1976.
4. Rivier, J. L., Monti, M., Mazzoni, S., Reymond, C., Jaggi, C. P. & Jaeger, M.: Le rôle du ralentissement de la fréquence cardiaque dans l'effet favorable des bêta-bloquants sur l'ischémie du myocarde provoquée par l'isoprénaline chez l'angineux. Schweiz Med Wochenschr 107: 1555, 1977.
5. Stephen, S. A.: Unwanted effects of propranolol. Am J Cardiol 18: 463, 1966.
6. Warren, V. & Goldberg, E.: Intractable angina pectoris. Combined therapy with propranolol and permanent pervenous pacemaker. JAMA 235: 841, 1976.

# Ullrich Noonan Syndrome

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STRACT A case of Ullrich Noonan syndrome h pulmonary stenosis epicanthus ptosis small ture curved tibia positive sex chromatin and a lo d chromosome number is presented A detailed omosomal band ng analysis with the G staining ita ning and Ag I staining techniques revealed sign ficant anomalies The l terature is reviewed d the criteria for diagnosing Ullrich Noonan syn ome are presented

y o ds Ullrich Noonan syndrome chromosome alys s cl n cal p cture

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llrich (25) described n 1930 an 8-year old g rl w th ebb ng of the neck small stature cub tus valgus om nent ears congen tal lymphang ectatic ede a ptos s dystrophy of the na ls hypoplast c n p- es triangular shaped mouth and small mand ble urner (24) reported n 1938 elderly female pat ents th webbed neck cub tus valgus and sexual nfan ism The sexual nfant l sm had not been appar it to Ullrich whose pat ents were young ch ldren urner had recognized the syndrome in females ly

When summariz ng ava lable informat on n 1949 llrich ( 6) noted a 4 1 preponderance of females ver males among the pat ents he had described in 930 He called the syndrome status Bonnev e Ull ch Bonnev e being a mouse geneticist work ng th a mutant strain of m ce w th webs of the neck well ng of the l mbs and other anomal es sugges ve of the phenotyp c f ndings of Ullrich s pa ents

These pat ents were described as having e ther onnevie Ullrich syndrome or Turner syndrome nt l the d scovery of sex chromat n by Barr & ertram (?) made a further d st nct on poss ble Vilk ns et al (?9) observed that many of these atients were chromat n negative and div ded the ond t on into chromatin pos t ve and chromatin negative Turner syndrome On clin cal grounds stress ng the important d scrim nating criterion of hypogonad sm Cafl sch (6) clearly separated Ull rich s syndrome from Turner s

After the report by Ford et al (9) of a chromosomal anomaly n a pat ent w th Turner syndrome this term became restricted to pat ents with webbing of the neck ovarian dysgenes s and other anomal es and also a 45 X chromosomal con stitut on The des gnat on Ullrich (or Bonnevie Ull rich) syndrome has been used to describe male and female pat ents w th the same phenotyp c features but with normal chromosomal constitut on (?1)

In 1963 Noonan & Ehmke (16) reported as soc ated non-cardiac malformat on in ch ldren with congen tal heart disease Further cases were added n 1968 (15) Pulmonary stenosis was the com monest although not the only cardiac malforma t on

This paper presents an nstance of Ullrich Noonan syndrome w th pulmonary stenos s ep canthus ptos s small stature curved tib a po tive sex chromatin and a diplo d chromosome number Furthermore to find out whether any cytogenet cal abnormalities were present an analysis by chromosome band ng techn ques was performed

## CASE REPORT

The pat ent s a 3? year-old woman whose mother was 37 years old at del ery Al hough the mother had no rubella and d d not smoke her p egnancy was compl cated by nausea vom t ng and t redness to such an extent that she was hosp tal zed al eady at the beg nning of he p egnan y and was gi en se eral drugs type unknown start ng dur ng the first month of p egnancy Her body we ght during pregnancy 50 kg was lower than normal (56 kg) and her he ght was 163 cm

The pat ent s a 3/3 s bl ng Her b rth we ght was 4930 g No congen tal malformat ons are known n her relat ves She has a congen tal b la eral p os s operated n 1953 and an ep canthus su g cally corrected n 1957 A heart catheterizat on in 1956 showed a normal p essure n the

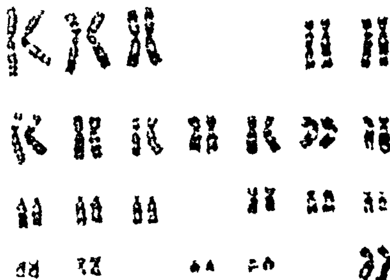


*Fig. 1.* G banded karyotype.

right atrium and pulmonary artery but elevated in the right ventricle 42 mmHg with a pressure gradient of 23 mmHg over the pulmonary artery valve. No signs of left-to-right shunt were observed.

Examination in 1961 at the age of 14 showed a body weight of 40.5 kg, height 151 cm. Corresponding values in 1976 were 47 kg and 160 cm. There were no signs of cardiac decompensation. No increased amount of nevi. Peripheral pulses were normal and blood pressure 115/80 mmHg. The left ventricular pulsations were normal whereas the right were slightly enhanced. A grade 4 (1–6) low pitched systolic murmur was heard in the left second interspace. X ray showed a slightly enlarged heart with a total volume of 640 ml, corresponding to a relative volume of 460 ml/m BSA (upper normal value 450) and a slight enlargement of the main pulmonary artery. Incomplete right bundle branch block was observed in the ECG. There was a ptosis and a ventrally convex bending of both tibiae. Apart from the small stature no further congenital malformations were observed, no webbed neck and no simian line. She is sex-chromatin positive.

Chromosome preparations were made by conventional air-drying from lymphocyte cultures. The slides were subjected to three different chromosome banding techniques, i.e. G staining (GTG), C staining (CBG) and the Ag I staining technique by incubation in $AgNO_3$-solution. The G staining technique of Wang and Fedoroff (28) and the C staining technique of Sumner (22) were used with minor modifications (12). Silver staining was essentially according to Bloom and Goodpasture (4).

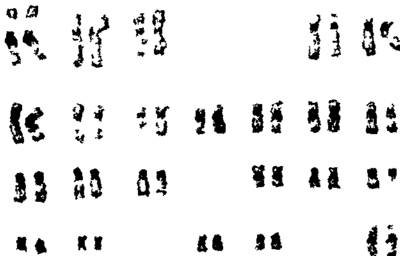


*Fig. 2.* C banded karyotype.

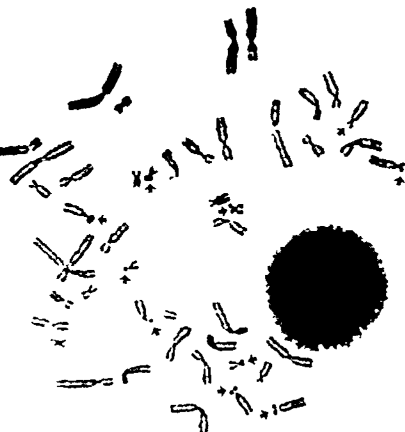


*Fig 3* Ag I stained metaphase cell Ag NORs are indicated by arrows

ll cells analysed had a normal diploid female yotype In the G banded metaphase no significant malous band pattern could be detected in any chromoe (*Fig 1*) *Similarly no abnormality in the distribution* he C bands (Fig 2) or in the localization of Ag NORs ; 3) was detectable No extreme size variant of C band present The modal number of Ag NORs was 9 al ugh 10 were observed in several cells

he patient became pregnant in 1968 The child died week after delivery from a Klebsiella sepsis Autopsy wed multiple malformations with hypertelorism penal oplasia phimosis and agenesis of septum pellucidum r next two pregnancies in 1971 and 1976 resulted in mal children The last pregnancy was complicated by fuse uterine bleedings and a hysterectomy was neces y Caesarian section was performed on all three occa ns due to narrow internal outlet of the pelvis Moderate idency to keloid formation was observed A missed ortion of a 7 cm long twin foetus was found at operation 1971

## DISCUSSION

ssociated non-cardiac malformation is not too un mmon in patients with congenital heart disease esen et al (5) reported an incidence of 11 4% in a clinical material comprising 615 children and of 13 4% in an autopsy material comprising 1 145 children below the age of four years excluding patients with Down s syndrome These authors did not find any definite correlation between a specific congenital heart disease and extra cardiac malformations although atrial septal defect seemed to be more common than expected in combination with harelip and cleft palate and ventricular septal defect with malformations of bones joints and lungs Noonan and Ehmke (16) examined 835 children with congenital heart disease and observed a total of 396 extra-cardiac anomalies in 242 patients (29%) In 68 instances more than one additional anomaly was noted The central nervous system was most frequently *involved followed in order by the mus* culo-skeletal genitourinary and gastrointestinal systems Noonan and Ehmke stressed the relation between a low birth weight and the presence of non cardiac anomalies Our patient with a birth weight of 4930 g exemplifies that this rule is not without exceptions

Pulmonary stenosis is the most common cardiac malformation in patients with Ullrich Noonan syndrome Nora et al (17) observed cardiovascular lesions in 45 of their 81 patients and 20 of these had pulmonary stenosis In a review of the literature Qazi et al (20) reached a similar figure 77 (50%) of 155 patients had pulmonary stenosis Atrial septal defect was most common in connection with pulmonary stenosis but could appear alone Other common lesions included coarctation of the aorta aortic stenosis patent ductus arteriosus and ventricular septal defect Eccentric hypertrophy of the left ventricle and progressive obstructive cardiomyopathy (1 8 11) congestive cardiomyopathy (3) and systolic prolapse of the mitral valve (23) have been reported in Ullrich Noonan syndrome

Siggers and Polani (21) compared their Ullrich Noonan syndrome patients with three randomly selected age and sex matched controls for each patient Pulmonary valve lesions affected 9 patients (45%) against 5 (7%) in the control group Other lesions that were seen more commonly in the patients with Ullrich s syndrome than in the controls were anomalous pulmonary venous drainage (9 vs 1 5%) persistent ductus arteriosus (22 vs 12%) coarctation of the aorta (18 vs 6%) and hypertrophic obstructive cardiomyopathy (9 vs 0%) Less common lesions in the patients compared with the controls were ventricular septal defect (18% i patients 37% in controls) congenital aortic stenosis (0 vs 11%) and Fallot s tetralogy (0 vs 11%)

According to the literature auditory defects are not a characteristic sign in Ullrich Noonan syndrome In a clinical material comprising 615 children with congenital heart lesions Melchior and Terslev (14) were unable to find a specific correlation between the different types of heart disease and the neurological disorders Single cases of pulmonary stenosis and congenital deafness have been reported (13) It should be mentioned that our patient had a slight hearing defect of neurogenic origin

The pulmonary stenosis in patients with Ullrich Noonan syndrome is often due to a dysplastic valve caused by an increased amount of unorganized myxomatous tissue Surgically obtained relief of the stenosis is often unsatisfactory Pulmonary artery branch stenosis involving many peripheral branches was common in the series reported by Nora et al (17) and other investigators

Although the nature and extent of the abnormalities in Ullrich Noonan syndrome suggest its association with chromosomal aberration normal karyotypes have been reported in almost all cases Minor chromosomal aberrations in three reported cases (10 19 27) have been claimed by Qazi et al (20) to be fortuitous rather than cause and effect related

Several later studies (7 17 18 20) have dealt with chromosome analysis mostly by conventional non differential staining but also by autoradiographic (18) and banding technique (17) Only one patient out of nearly 80 who have been karyotyped in these studies had an abnormal chromosome constitution (7) As the latter study was performed before the days of chromosome banding the characterization of the small extra acrocentric chromosome in this patient was incomplete The absence of such an extra chromosome in the other cases strongly indicates it to be coincidental with the Ullrich Noonan syndrome in this patient rather than causal However modern chromosome banding techniques (Q and G staining) were applied to 4 patients by Nora et al (17) in the search for minor chromosomal rearrangements In spite of the refinement inherent in these techniques they did not find any structural abnormalities

Three different banding techniques were applied to the chromosomes of our patient the G C and silver staining None of them revealed any kind of chromosomal rearrangement or peculiarities in the distribution of bands The G band pattern was perfectly normal the C bands were normally distributed and no extreme variants or heteromorphisms were present Also the Ag NORs were located in the expected sites and their number and size were within the normal range Thus at the level of chromosome analysis no deviations from a normal diploid female karyotype could be found this agrees with the findings of Nora et al (17)

Although solitary cases of Ullrich Noonan syndrome appear heredity seems to play a significant role in the aetiology almost every mode of genetic transmission has been proposed Transmission of the phenotype from father to son as reported by among others Quazi et al (20) is evidence against X linked transmission of the syndrome Nora et al (17) presented evidence consistent with an autosomal dominant mode of inheritance of the syndrome but with normal chromosomal constitutions One of our patient s children died early from a sep

...s but with multiple congenital lesions Her two ther children are healthy and show no signs of congenital lesions

The differential diagnosis against related syndromes might be difficult Nora et al (17) presented the following criteria as useful in diagnosing Ullrich Noonan syndrome Female or male Normal life expectancy except as modified by cardiovascular disease Small stature not invariable chromatin positive female — Neurological Intellectual development is fair to good but is usually below that of siblings occasional hearing loss — Head Characteristic facies narrow maxilla small mandible — Eyes Frequent epicanthic folds ptosis and *hypertelorism* — Ears Usually *prominent fleshy* posteriorly rotated and low set — Neck Webbed in about 50% of patients low posterior hairline Chest Shield shaped widely spaced hypoplastic nipples *breast development variable in females* — Cardiovascular Anomalies in approximately 35% pulmonic stenosis is most common coarctation of aorta rarely occurs Asymmetric septal hypertrophy and pulmonic branch stenosis frequently found — Extremities Cubitus valgus lymphedema of dorsum of hands and feet in infancy short fifth finger with clinodactyly — Urogenital Variable fertility Ovarian dysgenesis and infertility in others Cryptorchism in the usually infertile male Renal anomalies occur but are not common — Skeletal Pectus excavatum frequent scoliosis and kyphosis in about 20% absence of short fourth metacarpal bone — Skin and nails Pigmented nevi frequent marked tendency to keloid formation nails dystrophic short wide not convex — *Dermatoglyphics* Distal axial triradius ridge count not increased — *Incidence Undetermined but estimated* to be between 1 1000 and 1 2500

*Pulmonary valve stenosis is the lesion most* commonly seen in patients with Ullrich Noonan syndrome Congenital heart disease seems to be more frequent in these patients than in those with Turner s syndrome A normal sex chromatin and normal chromosomes for their phenotypic sex support a diagnosis of Ullrich Noonan syndrome

## REFERENCES


1 Baltaxe H A Levin A R Ehlers K H & Engle M A The appearance of the left ventricle in Noonan s syndrome Radiology 109 155 1973

2 Barr M L & Bertram E G A morphological distinction between neurones of the male and female and the behavior of the nuclear satellite during accelerated nucleoprotein synthesis Nature 163 676 1949

3 Battiste L E Feldt R H & Lie J T Congestive cardiomyopathy in Noonan s syndrome Mayo Clin Proc 52 661 1977

4 Bloom S E & Goodpasture C An improved technique for selective silver staining of nucleolar organizer *regions in human chromosomes* Hum Genet 34 199 1976

5 Boesen J Melchior J C Terslev E & Vendel S Extra-cardiac congenital malformation in children with congenital heart disease Acta Paediatr (Suppl 146 28 1963

6 Caflisch A Das Pterygium Thesis University of Zurich 1952

7 Celermajer J M Bowdler J D & Cohen D H Pulmonary stenosis in patients with the Turner phenotype in the male Am J Dis Child 116 351 1968

8 Ehlers K H Engle M A Levin A R & Deely W J Eccentric ventricular hypertrophy in familial and sporadic instances of 46XX XY Turner phenotype Circulation *45 639 1972*

9 Ford C E Jones K W Polani P E deAlmeida J C & Briggs J H A sex chromosome anomaly in a case of gonadal dysgenesis (Turner s syndrome) Lancet 1 711 1959

10 Heller R H The Turner phenotype in the male J Pediatr 66 48 1965

11 Hirsch H D Gelband H Garcia O Gottlieb S & Tamer D M Rapidly progressive obstructive *cardiomyopathy in infants with Noonan s syndrome* Report of two cases Circulation 52 1161 1975

12 Mandahl N & Fredga K Q G and C band patterns of the mink chromosomes Hereditas 81 211 1975

13 McEvoy J & Froggatt P Pulmonary stenosis and congenital deafness Lancet 1 660 1965

14 Melchior J C & Terslev E Diseases of the central nervous system in infants and children with congenital heart disease Dan Med Bull 2 41 1964

15 Noonan J A Hypertelorism with Turner phenotype A new syndrome with associated congenital heart disease Am J Dis Child 116 373 1968

16 Noonan J A & Ehmke D A Associated non-cardiac *malformation in children with congenital heart* disease J Pediatr 63 468 1963

17 Nora J J Nora A H Sinha A K Spangler R D & Lubs H A The Ullrich Noonan syndrome (Turner phenotype) Am J Dis Child 127 48 1974

18 Nora J J & Sinha A K Direct familial transmission of the Turner phenotype Am J Dis Child 116 343 1968

19 Oikawa K & Blizzard R Chromosomal studies of patients with congenital anomalies simulating those of gonadal aplasia N Engl J Med 264 1009 1961

20 Quazi Q H Arnon R G Paydar M H & Mapa H C Familial occurrence of Noonan syndrome Am J Dis Child 127 696 1974

21 Siggers D C & Polani P E Congenital heart disease in male and female subjects with somatic features of Turner s syndrome and normal sex chromo-

somes (Ullrich s and related syndromes) Br Heart J 34 41 1972
22 Sumner A T A simple technique for demonstrating centromeric heterochromatin Exp Cell Res 75 304 1972
23 Towne W D Fabian J S Rosen K M & Rahimtoola S H Systolic prolapse of the mitral valve in Noonan s syndrome Am Heart J 90/4 499 1975
24 Turner H H A syndrome of infantilism congenital webbed neck and cubitus valgus Endocrinology 23 566 1938
25 Ullrich O Uber typische Kombinationsbilder multipler Abartungen Z Kinderheilk 49 271 1930
26 — Turner s syndrome and status Bonnevie Ullrich A synthesis of animal phenogenetics and clinical observations on a typical complex of development anomalies Am J Hum Genet 1 179 1949
27 Urmenyi A M C Beattie M K & Mirza M R Turner s syndrome in a phenotypic male with XO/XY mosaicism and autosomal aberrations J Med Genet 3 220 1966
28 Wang H C & Fedoroff S Banding in human chromosomes treated with trypsin Nature New Biol 235 52 1972
29 Wilkins L Grumbach M M & van Wyk J J Chromosomal sex in ovarian agenesis J Clin Endocrinol 14 1270 1954

# LETTERS TO THE EDITOR

*Dear Sir*

In your issue 205 477 1979 Sivertsson Andersson and Hansson have published a study of the vascular adaptation to long term blood pressure reduction in patients with arterial hypertension treated either with a diuretic (me fruside) or a $\beta$ blocking agent (atenolol) Both the methods used and the conclusions drawn by the authors call for some comments

1 *Methodological aspects* In the result section and in the discussion the authors refer to blood flow and resistance (in the calf vessels) What they have measured is however *blood flow and blood pressure Vascular resistance* is a calculated figure that usually is quite uncertain and more like an index of flow and pressure In their article the authors furthermore use the forearm blood pressure measured with a cuff They calculated the mean pressure as diastolic BP plus one third of the pulse pressure To calculate mean vascular resistance in the calf from such an uncertain figure obtained from the forearm under the circumstances of the study cannot be scientifically sound Under the circumstances that the experiment entails both blood flow and the configuration of the pulse curve must have changed appreciably Even if it were admissible to use mean pressure calculated from cuff pressure as an indication of the true mean pressure under stable flow conditions the decreased pulsatile flow must have moved the true mean closer to the diastolic BP after reduction of BP with atenolol The mean values are thus not comparable and the calculated vascular resistance is meaningless It is also not clear to me whether the forearm BP really reflects the BP in the calf when the vessels are maximally dilated in the calf with markedly increased calf blood flow

2 *In view of the above reservations* the discussion of the results lacks any solid foundation but something of what is said above might explain why the present results do not coincide with the same authors *result when studying* the hand blood vessels by a similar technique

I must however take exception to the last sentence of the paper in which the authors after showing that BP *reduction does not give any change indicating reversibility* of structural vascular changes nevertheless state that hypertensive structural vascular changes in established human hypertension are partially but not completely reversible with BP lowering therapy

Yours sincerely
*Lars Werko* Sodertalje Sweden

*Dear Sir*

Reply to a letter from Lars Werko concerning our study published in the Acta Medica Scandinavia 205 477–482 1979

As Werko *points out the mean blood pressure in our* study was calculated as auscultatory diastolic blood pressure plus $^{1}/_{3}$ (factor $a$) of the pulse pressure In theory one might expect that $\beta$ blockers could change the shape of the pulse curve and thereby this factor In order to evaluate the possible role of such a change we compared simultaneously measured direct and indirect mean brachial *blood pressures in five patients before and after 6* weeks treatment with a $\beta$-blocker (propranolol 320 mg/day) Auscultatory mean pressure was calculated as diastolic blood pressure plus $^{1}/_{3}$ of the pulse pressure and intra arterial (i a ) mean pressure was obtained by electrical damping The calculated auscultatory mean pressure was significantly higher than the i a value under $\beta$ blockade but tended to be higher even without treatment (mean difference ± S D +7 4±3 5 and +4 8+8 7 mmHg respectively) The deviation of the calculated indirect mean pressure from the directly measured one was not significantly greater with than without $\beta$ blockade Furthermore factor $a$ calculated from i a systolic diastolic and electrically damped mean pressures was the same with and without $\beta$ blockers (0 37±0 05 and 0 38±0 04 mmHg respectively)

To evaluate the possible pressure drop between the arm and the leg we have compared i a blood pressure recordings in the brachial and femoral arteries The curves show very small differences and overlap almost completely both at rest and during exercise (1)

Thus although i a blood pressure recordings would be preferable we consider that mean blood pressure calculated from indirect blood pressures in the arm gives a good *estimation of the true mean pressure also under $\beta$ blockade* We also consider the mean blood pressure drop between the brachial and femoral arteries to be insignificant also during exercise induced hyperemia In conclusion we feel that all of Werko s comments have been refuted and that our conclusions are based on relevant data

Yours sincerely
*Ramon Sivertsson Ove Andersson Lennart Hansson*
East Hospital and Sahlgrenska Hospital
Gothenburg Sweden

## REFERENCE

1 Andersson O Management of hypertension Fig 2 *Acta Med Scand (Suppl) 617* 1978

*8 I Ann al Mect g of tle S c et f N lear Med c e* w ll take place in Erlangen/Nurnberg FRG Sept 9 17 1980

*I f r at on and s b n ss on of p pers* Congress Offce Prof Dr F Wolf Inst tut und Pol kl n k fur Nuklearmed z n Krankenhausstrasse 17 D 8500 Erlangen Germany

*NCI EORTC S pos m n Nat re Pre e o a d Treat ent of Cl n cal To c t f A cancer Ag nts* w ll be held n Brussels Belg um Sept 25–27 1980

*Reg strat ons d abs racts for f ee co n n cat s* ( n English 50–500 words) should be sent before Aug 1 1980 to Dr M St quet EORT Coord nator Inst tut Jules Bordet 1 rue Heger Bordet 1000 Brussels Belg um

*EORTC S pos u t on Ne Approacl es to Ca cer Tl erapy* to be held in Madrid Spa n Oct 1 3 1980 w ll focus on new anticancer agents and the role of com b ned modal t es in the treatment of adult and ch ldhood cancer

*Infor at n req ests and bstracts* ( 00–250 words by Aug 15 1980) should be addressed to Dr H Cortes Funes Hosp tal 1 de Octubre Carretera Andalucia km 5 5 Madrid 6 Spa n

*XIII C gress f tle E r pe n Acade y f All rg log nd Cl n l I* w ll be held n V enna Austria Oct 6–10 1980

*E q r es* Dr H Ludw g c/o W ener Mediz sche Akadem e Alser Straße 4 A 1090 V enna Austria

*F fth I t r t l Ped tr c Nepl r logy S p s* w ll be held n Ph ladelph a USA Oct 6–10 1980

*I f r t* Dr M E Norman Secretary General Room 6237 Ch ldren s Ho p tal of Ph ladelph a 34th and Civ c Center Boulevard Ph ladelph a Pennsylvan a 19104 USA

*Tl e Tl rd I ter t o l C gress o Obes ty* w ll take place in Rome Italy Oct 9 11 1980

*For f rtl er f r at n reg s rat d abst act for s c tact* Amer can Express Co S A I Conven t ons Serv ce Italy Piazza M gnanelli 4 I-00187 Roma Italy

# SUPPLEMENTS TO VOLUME 207



33–802986

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# LIST OF AUTHORS